IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

REDACTED
PUBLIC VERSION

BOSTON SCIENTIFIC CORPORATION and BOSTON SCIENTIFIC SCIMED, INC., Plaintiffs/Counter-Defendants, v. JOHNSON & JOHNSON, CORDIS CORPORATION, and WYETH Defendants/ Counter-Plaintiffs.	Civil Action No. 07-333-SLR Civil Action No. 07-348-SLR Civil Action No. 07-409-SLR
BOSTON SCIENTIFIC CORPORATION and BOSTON SCIENTIFIC SCIMED, INC., Plaintiffs/Counter-Defendants, v. JOHNSON & JOHNSON, CORDIS CORPORATION, and WYETH Defendants/Counter-Plaintiffs.	Civil Action No. 07-765-SLR

APPENDIX OF EXHIBITS TO DEFENDANTS/COUNTER-PLAINTIFFS JOHNSON & JOHNSON AND CORDIS'S OPPOSITION TO PLAINTIFFS' MOTIONS FOR SUMMARY JUDGMENT OF INVALIDITY PURSUANT TO 35 U.S.C.§ 103

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U.S. Patent No. 5,362,718		A1064-A1077
U.S. Patent No. 5,464,650		A1078-A1084
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U.S. Patent No. 5,516,781		A1091-A1097
U.S. Patent No. 5,545,208		A1098-A1111
U.S. Patent No. 5,563,145		A1112-A1122
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U.S. Patent No. 5,837,313		A1147-A1161
U.S. Patent No. 6,120,536		A1162-A1178
U.S. Patent No. 7,217,286		A1179-A1196
U.S. Patent No. 7,223,286		A1197-A1215
U.S. Patent No. 7,229,473		A1216-A1233

Document Description	Original Pagination	Appendix Pagination
U.S. Patent No. 7,300,662		A1234-A1257
Brack *et al.*, The Subcutaneous Heparin and Angioplasty Restenosis Prevention (SHARP) Trial, *J Am Coll Cardiol* 1995;26.	947-954	A1258-A1266
Fischman, *et al.*, A Randomized Comparison of Coronary-Stent Placement and Balloon Angioplasty in the Treatment of Coronary Artery Disease, *New J of Med* 1994;331.	496-501	A1267-1272
Honda *et al.*, Intravascular Ultrasound, in *Textbook of Interventional Cardiology*, (Eric J. Topol ed., 5th ed. 2008)	1115-1119, 1125-1132	A1273-A1287
Leaf *et al.*, Do Fish Oils Prevent Restenosis after Coronary Angioplasty, *Circulation* 1994;90.	2248-2257	A1288-A1298
Abizaid *et al.*,Randomized, Double-Blind, Multicenter Study of the Polymer-Based 17- β Estradiol-Eluting Stent for Treatment of Native Coronary Artery Lesions: Six-Month Results of the ETHOS I Trial, *Catheter Cardiovasc Interv* 2007;70.	654-660	A1299-A1305
American College of Cardiology 52nd Annual Scientific Session, *Scientific Session News* 2003;21(6).	1-8	A1306-A1313
Adamian *et al.*, Cutting Balloon Angioplasty for the Treatment of In-Stent Restenosis: A Matched Comparison With Rotational Atherectomy, Additional Stent Implantation and Balloon Angioplasty, *J Am Coll Cardiol* 2001;38.	672-679	A1314-A1323
Adelman *et al.*, A Comparison of Directional Atherectomy with Balloon Angioplasty for Lesions of the Left Anterior Descending Coronary Artery, *N Engl J Med* 1993;329.	228-233	A1324-A1330
The American Heart Association, *Top Ten Research Advances in 2003*, http://www.americanheart.org/ presenter.jhtm?identifier=3052793.		A1331-A1337
Number Not Used		A1338-A1344
The American Heart Association, *Top Ten Research Advances for 2001*, http://www.americanheart.org/ presenter.jhtm?identifier=3000094.		A1345-A1360

Document Description	Original Pagination	Appendix Pagination
Lawrence K. Altman, Device for Clogged Arteries Gets a Boost, *N.Y. Times*, Jan. 16, 2001, at F8.		A1361-A1363
Appelman *et al.*, Randomised Trial of Excimer Laser Angioplasty Versus Balloon Angioplasty for Treatment of Obstructive Coronary Artery Disease, *Lancet* 1996;347.	79-84	A1364-A1370
Armstrong *et al.*, Angiopeptin-Eluting Stents: Observations in Human Vessels and Pig Coronary Arteries. *J of Invasive Cardiol* 2002;14(5).	230-238	A1371-A1379
Bejarano *et al.*, In-stent Restenosis. Acute and Long-Term Outcomes after Excimer Laser Coronary Angioplasty, *Arq Bras Cardiol* 1999;73(2).	153-156	A1380-A1383
Berg *et al.*, Pimecrolimus and Dual Pimecrolimus-Paclitaxel Eluting Stents Decrease Neointimal Proliferation in a Porcine Model, *Catheter Cardiovasc Interv* 2007;70.	871-879	A1384-A1393
Bertrand *et al.*, Effect of Pravastatin on Angiographic Restenosis after Coronary Balloon Angioplasty, *J Am Coll Cardiol* 1997;4.	863-869	A1394-A1401
Braun *et al.*, Rotablator Versus Cutting Balloon for the Treatment of Long In-Stent Restenoses, *J Invas Cardiol* 2002;14.	291-296	A1402-A1409
David Brieger and Eric Topol, Local Drug Delivery Systems and Prevention of Restenosis, *Cardio Res* 1997;35(3).	405-413	A1410-A1418
Press Release, Guidant Corp., Guidant Halts Further Development on Actinomycin-D Drug Eluting Stent Program (Mar. 7, 2002).		A1419-A1420
Press Release, Guidant Corp., Guidant Halts Further Development on Actinomycin-D Drug Eluting Stent Program (Mar. 7, 2002) (Ex. PX 112).		A1421-A1422
Press Release, Guidant Corp., Guidant Reports Preliminary Results of DELIVER Clinical Trial (Jan. 2, 2003).		A1423-A1424

Document Description	Original Pagination	Appendix Pagination
Press Release, Guidant Corp., Guidant Reports Preliminary Results of DELIVER Clinical Trial (Jan. 2, 2003) (Ex. PX 113).		A1425-A1426
Burchenal *et al.*, Effect of direct thrombin inhibition with bivalirudin (Hirulog) on restenosis after coronary angioplasty, *Am J Cardiol* 1998;82.	511-515	A1427-A1432
Cairns *et al.*, Fish Oils and Low-molecular-weight Heparin for the Reduction of Restenosis after Percutaneous Transluminal Coronary Angioplasty, *Circulation* 1996;94.	1553-1560	A1433-A1441
REDACTED		
Carter *et al.*, Long-term effects of polymer-based, slow-release, sirolimus-eluting stents in a porcine coronary model, *Cardio Res* 2004;63.	617-624	A1444-A1451
Chevalier *et al.*, A Randomized, Controlled, Multicenter Trial to Evaluate the Safety and Efficacy of Zotarolimus-Versus Paclitaxel-Eluting Stents in De Novo Occlusive Lesions in Coronary Arteries: The ZoMaxx I Trial, *JACC: Cardiovascular Interventions,* 2008;1(5).	524-532	A1452-A1462
Cleveland Clinic, Coronary Artery Disease – Risk factors, http://my.clevelandclinic.org/heart/prevention/riskfactors.aspx		A1463-A1468
Dangas *et al.*, Restenosis: Repeat Narrowing of a Coronary Artery: Prevention and Treatment, *Circulation* 2002;105.	2586-87	A1469-A1470
Darius *et al.*, Effects of Ciprostene on Restenosis Rates During Therapeutic Transluminal Coronary Angioplasty, *Agents Actions Suppl* 1992;37.	305-311	A1471-A1478
Dawkins *et al.*, Clinical Efficacy of Polymer-Based Paclitaxel-Eluting Stents in the Treatment of Complex, Long Coronary Artery Lesions From a Multicenter, Randomized Trial: Support for the Use of Drug-Eluting Stents in Contemporary Clinical Practice, *Circulation* 2005;112.	3306-3313	A1479-A1487

Document Description	Original Pagination	Appendix Pagination
De Scheerder *et al.*, Dexamethasone: mode of action, preclinical, and clinical studies, in *Handbook of Drug-Eluting Stents* (Patrick W. Serruys & Anthony H. Gershlick eds., 2005).	273-283	A1488-A1504
Number Not Used		A1505-A1521
Degertekin *et al.*, Persistent Inhibition of Neointimal Hyperplasia After Sirolimus-Eluting Stent Implantation Long Term (Up to 2 Years) Clinical, Angiographic, and Intravascular Ultrasound Follow-Up, *Circulation* 2002; 106.	1610-1613	A1522-A1526
Desmet *et al.*, Angiotensin-converting Enzyme Inhibition with Fosinopril Sodium in the Prevention of Restenosis after Coronary Angioplasty. *Circulation* 1994;89.	385-392	A1527-A1535
Emanuelsson *et al.*, Long-term Effects of Angiopeptin Treatment in Coronary Angioplasty. Reduction of Clinical Events but not Angiographic Restenosis, *Circulation* 1995; 91.	1689-1696	A1536-A1544
The ERASER Investigators, Acute Platelet Inhibition with Abciximab Does Not Reduce In-Stent Restenosis (ERASER Study), *Circulation* 1999;100.	799-806	A1545-A1553
Fajadet *et al.*,Randomized, Double-Blind, Multicenter Study of the Endeavor Zotarolimus-Eluting Phosphorylcholine-Encapsulated Stent for Treatment of Native Coronary Artery Lesions: Clinical and Angiographic Results of the ENDEAVOR II Trial, *Circulation* 2006;114.	798-806	A1554-A1563
Faxon, Effect of High Dose Angiotensin-Converting Enzyme on Restenosis, *J Am Coll Cardiol* 1995;25.	362-369	A1564-A1572
Faxon *et al.*, Low Molecular Weight Heparin in Prevention of Restenosis after Angioplasty, *Circulation* 1994;90.	908-914	A1573-A1579
Fischell, Polymer Coatings for Stents: Can We Judge a Stent by Its Cover?, *Circulation* 1996;94.	1494-1495	A1580-A1582
Fischman *et al.*, A Randomized Comparison of Coronary-Stent Placement and Balloon Angioplasty in the Treatment of Coronary Artery Disease, *New Eng. J. Med.*1994;331.	496-501	A1583-A1588

Document Description	Original Pagination	Appendix Pagination
Freed *et al.*,Combination of lovastatin, enalapril, and colchicine does not prevent restenosis after percutaneous transluminal coronary angioplasty. *Am J Cardiol* 1995;76.	1185-1188	A1589-A1593
REDACTED		
Garas *et al.*, Overview of therapies for prevention of restenosis after coronary interventions, *Pharmacol Ther* 2001; 92.	165-178	A1609-A1622
Gershlick *et al.*, Failure of Epoprostenol (Prostacyclin, PGI2) to Inhibit Platelet Aggregation and to Prevent Restenosis after Coronary Angioplasty: Result of a Randomized Placebo Controlled Trial, *Br Heart J* 1994;71.	7-15	A1623-A1631
Gibson *et al.*, Six-month Angiographic and Clinical Follow-up of Patients Prospectively Randomized to Receive either Tirofiban or Placebo during Angioplasty in the RESTORE Trial, *J Am Coll Cardiol* 1998;32.	28-34	A1632-A1639
Gimple *et al.*, Usefulness of Subcutaneous Low Molecular Weight Heparin (Ardeparin) for Reduction of Restenosis after Percutaneous Transluminal Coronary Angioplasty, *Am J Cardiol* 1999; 83.	1524-1529	A1640-A1647
Grube *et al.*, High-dose 7-hexanoyltaxol-eluting stent with polymer sleeves for coronary revascularization One-year results from the SCORE randomized trial, *J Am Coll Cardiol* 2004;44.	1368-1372	A1648-A1654
Gruberg, BRILLIANT-EU: Batimastat (BB94) Antirestenosis Trial using the BiodivYsio Local Drug Delivery PC-Stent, *Euro Society of Cardiol* 2003, http://www.medscape.com/viewarticle/461343.		A1655-A1658

Document Description	Original Pagination	Appendix Pagination
Gruberg, ENDEAVOR I: Safety and Efficacy of the ABT-578-Coated Endeavor Stent – 12-Month Follow-up, *Medscape Cardiology* 2004, http://www.medscape.com/ viewarticle/782829.		A1659-A1661
Grube *et al.*, SCORE Trial Interim Safety Results: Despite Efficacy, Late Stent Thrombosis With The QuaDDS-QP2 Stent. *J Am Coll Cardiol* 2002;39 (suppl. A).	38A	A1662
Grube *et al.*,TAXUS I: Six- and Twelve-Month Results From a Randomized, Double-Blind Trial on a Slow-Release Paclitaxel-Eluting Stent for De Novo Coronary Lesions, *Circulation* 2002;107.	38-42	A1663-A1668
Gunn and Cumberland, Stent coatings and local drug delivery, *Euro Heart J* 1999;20.	1693-1700	A1669-A1677
Haase *et al.*, Acute and One Year Follow-Up Results after Vessel Size Adapted PTCA Using Intracoronary Ultrasound, *Euro Heart J* 1998;19.	263-272	A1678-A1687
Hoberg *et al.*, Prevention of Restenosis after PTCA: Role of Calcium Antagonists, *J Cardiovasc Pharmacol* 1991;18.	S15-S19	A1688-A1693
Hoffmann *et al.*, Evaluation of a High-Dose Dexamethasone-Eluting Stent, *Am J Cardiol* 2004;94.	193-195	A1694-A1696
Holmes *et al.*, A Multicenter, Randomized Trial of Coronary Angioplasty Versus Directional Atherectomy for Patients with Saphenous Vein Bypass Graft Lesions, *Circulation* 1995;91.	1966-1974	A1695-A1707
Holmes *et al.*,Results of Prevention of Restenosis with Tranilast and its Outcomes (PRESTO) Trial, *Circulation* 2002;106.	1243-1250	A1708-A1716
Izumi *et al.*, Final Results of the CAPAS Trial, *Am Heart J* 2001; 142	782-787	A1717-A1726
Jorgensen *et al.*, Restenosis and Clinical Outcomes in Patients Treated with Amlodipine after Angioplasty; Results from the Coronary AngioPlasty Amlodipine Restenosis Study (CAPARES), *J Am Coll Cardiol* 2000; 53.	592-599	A1727-A1735

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Karsch *et al.*, Low Molecular Weight Heparin (Reviparin) in Percutaneous Transluminal Coronary Angioplasty, *J Am Coll Cardiol* 1996;28.	1437-1443	A1736-A1743
Kimura *et al.*, Three-Year Follow-Up After Implantation of Metallic Coronary Artery Stents, *N. Engl. J. Med.* 1996;334.	561-66	A1744-A1749
Number Not Used		A1750-A1755
REDACTED		
Kondo *et al.*, Immediate and Chronic Results of Cutting Balloon Angioplasty: A Matched Comparison with Conventional Angioplasty, *Clin Cardiol* 1997;20.	459-463	A1763-A1768
Kondo *et al.*, Effect of Quinapril on Intimal Hyperplasia After Coronary Stenting as Assessed by Intravascular Ultrasound, *Am J Cardiol* 2001;87.	443-445	A1769-A1773
Krucoff *et al.*, A Novel Bioresorbable Polymer Paclitaxel-eluting Stent for the Treatment of Single and Multivessel Coronary Disease: Primary Results of the COSTAR (Cobalt Chromium Stent With Antiproliferative for Restenosis) II Study, *J Am Coll Cardiol* 2008;51.	1543-1552	A1774-A1783
Kutryk, M. Drug-eluting stents for the treatment of coronary artery disease Part 4: New results from clinical trials and future directions. *Cardiol Rounds* 2003;VIII (10).		A1784-A1789
Kutryk, Drug-eluting stents for the treatment of coronary artery disease Part 3: New results from clinical trials, *Cardiol Rounds* 2003;VIII (9).		A1790-A1795
Lablanche *et al.*, Effect of the Direct Nitric Oxide Donors Linsidomine and Molsidomine on Angiographic Restenosis after Coronary Balloon Angioplasty, *Circulation* 1997;95.	83-89	A1796-A1803

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Lablanche *et al.*, Effect of Nadroparin, a Low-molecular-weight Heparin, on Clinical and Angiographic Restenosis after Coronary Balloon Angioplasty: the FACT Study, *Circulation* 1997;96.	3396-3402	A1804-A1811
Antoine Lafont, The Cypher stent: no longer efficacious at three months in the porcine model?, *Cardio Res* 2004;63.	575-576	A1812-A1813
Lansky *et al.*, Non-Polymer-Based Paclitaxel-Coated Coronary Stents for the Treatment of Patients With De Novo Coronary Lesions: Angiographic Follow-Up of the DELIVER Clinical Trial, *Circulation* 2004;109.	1948-1954	A1814-A1821
Liu *et al.*, Batimastat (BB-94) anti-restenosis trial utilizing the BiodivYsio local drug delivery PC-stent (BRILLIANT-EU-trial). In: ESC Congress 2003 Abstract (Vienna, Austria, Aug 30-Sept 3, 2003).		A1822
Mauri L *et al.,* Late Loss in Lumen Diameter and Binary Restenosis for Drug-Eluting Stent Comparison, *Circulation* 2005;111.	3435-3442	A1823-A1831
Number Not Used		A1832-A1840
Mauri L *et al.,* Relationship of Late Loss in Lumen Diameter to Coronary Restenosis in Sirolimus-Eluting Stents, *Circulation* 2005;111.	321-327	A1841-A1848
Mauri *et al.*, Robustness of Late Lumen Loss in Discriminating Drug-Eluting Stents Across Variable Observational and Randomized Trials, *Circulation* 2005; 112.	2833-2839	A1849-A1856
McClean *et al.*, The importance of the drug platform: coated, uncoated, sleeves, and new concepts in *Handbook of Drug-Eluting Stents* (Serruys P Gershlick A (eds.) (2005).	75-84	A1857-A1868
Mehran *et al.*, In-Stent Restenosis: "The Great Equalizer" – Disappointing Clinical Outcomes with ALL Interventional Strategies, *J Am Coll Cardiol* 1999.	63A	A1869-A1870
The MERCATOR Study Group, Does the New Angiotensin Converting Enzyme Inhibitor Cilazapril Prevent Restenosis After Percutaneous Transluminal Coronary Angioplasty?, *Circulation* 1992;86.	100-110	A1871-A1881

Document Description	Original Pagination	Appendix Pagination
Miyazawa *et al.,*Comparison of vascular response to zotarolimus-eluting stent versus sirolimus-eluting stent: Intravascular ultrasound results from ENDEAVOR III, *Am Heart J* 2008;155.	108-13	A1882-A1892
Moreno *et al.*, Clinical Impact of In-Stent Late Loss after Drug-Eluting Coronary Stent Implantation, *Euro Heart J* 2007; 28.	1583-91	A1893-A1901
Morgan Stanley Industry Overview (February 24, 2005)	1-34	A1902-A1935
Morice *et al.,* A Randomized Comparison Of A Sirolimus-Eluting Stent With A Standard Stent For Coronary Revascularization, *N Engl J Med* 2002;346(23).	1773-1780	A1936-A1944
Moses *et al.,* Sirolimus-eluting Stents versus Standard Stents in Patients with Stenosis in Native Coronary Artery, *N Engl J Med* 2003;349.	1315-1323	A1945-A1953
New *et al.,*Estrogen-Eluting, Phosphorylcholine-Coated Stent Implantation Is Associated With Reduced Neointimal Formation But No Delay in Vascular Repair in a Porcine Coronary Model, *Catheter Cardiovasc Interv* 2002;57.	266-271	A1954-A1968
Norell *et al.,* Essential Interventional Cardiology 68 (2d ed. 2008).	63-79	A1969-A1992
New Stent Is Hailed in Cardiac Treatment, *The New York Times* (Sept. 5, 2001), C15.		A1993
O'Keefe *et al.,* Effects of Diltiazem on Complications and Restenosis after Coronary Angioplasty, *Am J Cardiol* 1991; 67.	373-376	A1994-A1998
O'Keefe *et al.,* Ineffectiveness of Colchicine for the Prevention of Restenosis after Coronary Angioplasty, *Am J Cardiol* 1992;67.	1597-1600	A1999-A2003
O'Keefe *et al.,* Lovastatin Plus Probucol for Prevention of Restenosis after Percutaneous Transluminal Coronary Angioplasty, *Am J Cardiol* 1996;77.	649-652	A2004-A2008

Document Description	Original Pagination	Appendix Pagination
Okumura *et al.*, Quinapril Prevents Restenosis after Coronary Stenting in Patients with Angiotensin-Converting Enzyme D Allele, *Circ J* 2002;66.		A2009-A2014
O'Neill *et al.*, Long-term Treatment with a Platelet Glycoprotein-receptor Antagonist after Percutaneous Coronary Revascularization, *N Engl J Med* 2000;342.	1316-1324	A2015-A2024
Orloff *et al.*, Biodegradable implant strategies for inhibition of restenosis, *Advanced Drug Delivery Reviews* 1997;24.	3-9	A2025-A2031
Pepine *et al.*, A Controlled Trial of Corticosteroids to Prevent Restenosis after Coronary Angioplasty, *Circulation* 1990;81.	1753-1761	A2032-A2040
Pocock *et al.*, Angiographic Surrogate End Points in Drug-Eluting Stent Trials. *J Am Coll Cardiol* 2008;51.	23-32	A2041-A2052
REDACTED		
Popma *et al.*, Qualitative and Quantitative Coronary Angiography, in *Textbook of Interventional Cardiology* (Eric J. Topol ed., 5th ed. 2008).	1071-1093	A2068-A2090
Raizner *et al.*, Inhibition of Restenosis with Beta-Emitting Radiotherapy: Report of the Proliferation Reduction With Vascular Energy Trial (PREVENT), *Circulation* 2000; 102.	951-958	A2091-A2099
Rechavia *et al.*, Biocompatibility of polyurethane-coated stents: tissue and vascular aspects, *Catheterization and Cardiovascular Diagnosis* 1998; 45(2).	202-207	A2100-A2105
Regar, *et al.* Angiographic Findings of the Multicenter Randomized Study With the Sirolimus-Eluting Bx Velocity Balloon-Expandable Stent (RAVEL): Sirolimus-Eluting Stents Inhibit Restenosis Irrespective of the Vessel Size, *Circulation* 2002;106.	1949-1956	A2106-A2114
Rensing *et al.*, Coronary restenosis elimination with a sirolimus-eluting stent: first European human experience with 6-month angiographic and intravascular ultrasonic follow-up. *Eur Heart J* 2001;22.	2125-30	A2115-A2120

Document Description	Original Pagination	Appendix Pagination
Savage *et al.*, Effect of Thromboxane A2 Blockade on Clinical Outcome and Restenosis after Successful Coronary Angioplasty, *Circulation* 1995;92.	3194-3200	A2121-A2128
Schampaert *et al.,* The Canadian Study of the Sirolimus-Eluting Stent in the Treatment of Patients With Long De Novo Lesions in Small Native Coronary Arteries (C-SIRIUS), *J Am Coll Cardiol* 2004;43.	1110-1115	A2129-A2134
Schofer *et al.*, Sirolimus-eluting stents for treatment of patients with long atherosclerotic lesions in small coronary arteries: double-blind, randomised controlled trial (E-SIRIUS). *The Lancet* 2003;362.	1093-1099	A2135-A2141
Schwartz *et al.,* Aspirin and Dipyridamole in the Prevention of Restenosis after Percutaneous Transluminal Coronary Angioplasty, *N Engl J Med* 1988;318.	1714-1719	A2142-A2148
REDACTED		
Schwartz *et al.*, Restenosis After Balloon Angioplasty, A Practical Proliferative Model in Porcine Coronary Arteries, *Circulation*, 1990; 82(6).	2190-2200	A2156-A2181
Serruys *et al.*, A Comparison of Hirudin with Heparin in the Prevention of Restenosis after Coronary Angioplasty, *N Engl J Med* 1995;333.	757-763	A2182-A2198
Serruys *et al.*, A randomized placebo-controlled trial of fluvastatin for prevention of restenosis after successful coronary balloon angioplasty, *Euro Heart J* 1999;20.	58-69	A2199-A2211
Serruys, *et al.*, Actinomycin-eluting stent for coronary revascularization: A randomized feasibility and safety study: The ACTION trial, *J Am Coll Cardiol* 2004;44.	1363-1367	A2212-A2219
Serruys *et al.,* Carvedilol for Prevention of Restenosis after Directional Coronary Atherectomy, *Circulation* 2000;1001.	1512-1518	A2220-A2232
Serruys *et al.*, Evaluation of Ketanserin in the Prevention of Restenosis after Percutaneous Transluminal Coronary Angioplasty, *Circulation* 1993;88.	1588-1601	A2233-A2246

Document Description	Original Pagination	Appendix Pagination
Serruys *et al.*, Prevention of Restenosis after Percutaneous Transluminal Coronary Angioplasty with Thromboxane A2-receptor Blockade. A Randomized, Double-Blind, Placebo-controlled Trial. *Circulation* 1991;84.	1568-1580	A2247-A2260
Serruys *et al.*, Rapamycin eluting stent: the onset of a new era in interventional cardiology, *Heart* 2002 April; 87(4).	305-307	A2261-A2263
Serruys *et al.*, A randomized comparison of a durable polymer Everolimus-eluting stent with a bare metal coronary stent: The SPIRIT first trial, *EuroIntervention* 2005;1.	58-65	A2264-A2271
Serruys *et al.*,The TRAPIST Study: A multicentre randomized placebo controlled clinical trial of trapidil for prevention of restenosis after coronary stenting, measured by 3-D intravascular ultrasound, *Eur Heart J* 2001; 22.	1938-1947	A2272-A2281
Serruys, *et al.*, Vascular Responses at Proximal and Distal Edges of Paclitaxel-Eluting Stents: Serial Intravascular Ultrasound Analysis From the TAXUS II Trial, *Circulation* 2004;109.	627-633	A2282-A2289
Slavin *et al.*, Drug-Eluting Stents: Preventing Restenosis, *Cardiology in Review* 2007;15.	1-12	A2290-A2301
Smock, Medical Miracles, *Design News* (Aug. 14, 2005).		A2302-A2303
Sousa *et al.*, Lack of Neointimal Proliferation After Implantation Of Sirolimus-Coated Stents in Human Coronary Arteries: A Quantitative Coronary Angiography and Three-Dimensional Intravascular Ultrasound Study, *Circulation* 2001;103.	192-195	A2304-A2310
Sousa, Pioneer clinical experiences: from bare metal Palmaz-Schatz™ to Sirolimus-Eluting stents, *EuroIntervention* 2006;2.	37-39	A2311-A2313
Sousa *et al.*, Sustained Suppression of Neointimal Proliferation by Sirolimus-Eluting Stents: One-Year Angiographic and Intravascular Ultrasound Follow-Up, *Circulation* 2001;104.	2007-2011	A2314-A2319

Document Description	Original Pagination	Appendix Pagination
Stone *et al.*, Comparison of an Everolimus-Eluting Stent and a Paclitaxel-Eluting Stent in Patients With Coronary Artery Disease: A Randomized Trial. *J Am Med Assn* 2008;299.	1903-1913	A2320-A2331
Stone *et al.*, A Polymer-Based, Paclitaxel-Eluting Stent in Patients With Coronary Artery Disease, *N Engl J Med* 2004; 350.	221-231	A2332-A2342
REDACTED		
Swain, Blazing New Paths for Products Introductions, *Medical Device and Diagnostic Industry* 2003.		A2362-A2369
Tanabe *et al.*,TAXUS III Trial: In-Stent Restenosis Treated With Stent-Based Delivery of Paclitaxel Incorporated in a Slow-Release Polymer Formulation, *Circulation* 2003;107.	559-564	A2370-A2376
TCT Daily: ZOMAXX I – Mixed Results with Zotarolimus-Eluting Stent, *J of Interventional Cardiol* 2007;20.	35	A2377
Teirstein, Living the Dream of No Restenosis, *Circulation* 2001;104.	1996-1998	A2378-A2380
Teirstein *et al.*, Three-year Clinical and Angiographic Follow-up after Intracoronary Radiation: Results of a Randomized Clinical Trial, *Circulation* 2000;101.	360-365	A2381-A2387
Thornton *et al.*, Coumadin and Aspirin in Prevention of Recurrence after Transluminal Coronary Angioplasty, *Circulation* 1984;69.	721-726	A2388-A2395
Topol *et al.*, Analysis of coronary angioplasty practice in the United States with an insurance-claims data base. *Circulation* 1993;87.	1489-1497	A2396-A2405
Topol *et al.*, A Comparison of Directional Atherectomy with Coronary Angioplasty in Patients with Coronary Artery Disease, *N Engl J Med* 1993;329.	221-227	A2406-A2413

Document Description	Original Pagination	Appendix Pagination
Topol *et al.*, Randomised Trial of Coronary Intervention with Antibody Against Platelet IIIb/IIIa Integrin for Reduction of Clinical Restenosis: Results at Six Months, The EPIC Investigators. *Lancet* 1994;343.	881-885	A2414-A2421
Tsuchida *et al.*, One-year results of a durable polymer everolimus-eluting stent in *de novo* coronary narrowings (The SPIRIT FIRST Trial), *EuroIntervention* 2005;1.	266-272	A2422-A2428
Urban *et al.*, Lack of Effect of Warfarin on the Restenosis Rate or on Clinical Outcome after Balloon Coronary Angioplasty, *Br Heart J* 1988;60.	485-488	A2429-A2434
van der Giessen *et al.*, Marked Inflammatory Sequelae to Implantation of Biodegradable and Nonbiodegradable Polymers in Porcine Coronary Artieries, *Circulation* 1996;94(7).	1690-1697	A2435-A2442
Verheye *et al.*, The GENESIS (Randomized, Multicenter Study of the Pimecrolimus-Eluting and Pimecrolimus/Paclitaxel-Eluting Coronary Stent System in Patients with De Novo Lesions of the Native Coronary Arteries) Trial, *J Am Coll Cardiol* 2005;2.	205-214	A2443-A2454
vom Dahl *et al.*, Clinical and Angiographic Predictors of Recurrent Restenosis after Percutaneous Transluminal Rotational Atherectomy for Treatment of Diffuse In-Stent Restenosis, *Am J Cardiol* 1999;83.	862-867	A2455-A2461
Waksman *et al.*, Late total occlusion after intracoronary brachytherapy for patients with in-stent restenosis, *J Am Coll Cardiol* 2000;36.	65-68	A2462-A2467
Weintraub *et al.*, Lack of Effect of Lovastatin on Restenosis after Coronary Angioplasty, *N Engl J Med* 1994;331(20).		A2468-A2483
Whitworth *et al.*, Effect of Nifedipine on Recurrent Stenosis after Percutaneous Transluminal Coronary Angioplasty, *J Am Coll Cardiol* 1986;8.	1271-1276	A2484-A2490
Wilczek *et al.*, Comparison of self-expanding polyethylene terephthalate and metallic stents implanted in porcine iliac arteries, *Cardiovascular Intervent Radiol* 1996;19.	176-180	A2491-A2495

Document Description	Original Pagination	Appendix Pagination
Number Not Used		A2496-A2500

REDACTED

Document Description	Original Pagination	Appendix Pagination
REDACTED		
Curriculum Vitae of Antonios G. Mikos (Exhibit A from Mikos' Responsive Expert Report of July 14, 2009)	1-134	A2671-A2993
REDACTED		
Abbott Laboratories, *Form 10-K* (2008).	1-107	A2998-A3107
REDACTED		
Bernstein Research Call, Medical Devices (Aug. 16, 2001).	1-3	A3123-A3125
Number Not Used		A3126-A3131
REDACTED		
Number Not Used		A3137-A3138
REDACTED		
Merrill Lynch Interventional Cardiology Report (July 31, 2003).	1-6	A3266-A3271
Morgan Stanley Industry Overview (Feb. 24, 2005).	1-33	A3272-A3305
REDACTED		
Number Not Used		A3322-A3355
REDACTED		

Document Description	Original Pagination	Appendix Pagination
REDACTED		
Non-Infringement Claim Charts for '7286, '3286, '473 (Exs. 9-11)	1-6; 1-14; 1-7	A3403-A3429
Claim Charts for '7286, '473, '286, '662	1-23	A3430-3452
Promus™ Everolimus Eluting Coronary Stent System Instructions for Use	1-60	A3453-A3512
Medtronic Coronary Stent Systems, Clinical Programs, (Apr. 2009).	1-29	A3513-A3544
ENDEAVOR® Zotarolimus-Eluting Coronary Stent System, Over-the-Wire Delivery System Instructions for Use.	1-48	A3545-A3594
Reply Pursuant to 37 CFR 1.1.11 by Maurice Valla to Examiner Sharon Kennedy re: Drug/Drug Delivery Systems for the Prevention and Treatment of Vascular Disease. Appl. No. 10/829,074 (Jan. 8, 2007).	1-9	A3595-A3603
XIENCE™ V Everolimus Eluting Coronary Stent System Instructions for Use.	1-60	A3604-A3663
Xience PMA – Section 5.0 Xience V Everolimus Eluting Coronary Stent System Description.	5-1 – 5-28	A3664-A3691
De Scheerder *et al.*, Batimastat: mode of action, preclinical, and clinical studies, in *Handbook of Drug-Eluting Stents* (Patrick W. Serruys & Anthony H. Gershlick eds., 2005).	247-259	A3692-A3710
Serruys *et al.*, A Comparison of Balloon-Expandable-Stent Implantation with Balloon Angioplasty Patients with Coronary Disease, *New Eng. J. Med.* 1994;331.	489-495	A3711-A3717
REDACTED		

Document Description	Original Pagination	Appendix Pagination
REDACTED		
Press Release, Guidant Corp., Guidant Reports Preliminary Results of Deliver Clinical Trial (Jan. 2, 2003) (Ex. PX 113)		A3879-A3880
Press Release, Guidant Corp., Guidant Halts Further Development on Actinomycin-D Drug Eluting Stent Program (Mar. 7, 2002) (Ex. PX 112).		A3881-A3882
U.S. Patent No. 6,746,773		A3883-A3895
REDACTED		

A1-166

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(54) Stent with local rapamycin delivery

Stent mit lokaler Rapamycin-Zufuhr

Stent permettant l'administration locale de rapamycine

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(56) References cited:
EP-A- 0 761 251
EP-A- 0 938 878
WO-A-98/47447
US-A- 5 700 286
US-A- 5 728 150

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Description

[0001] Delivery of rapamycin locally, particularly from an intravascular stent, directly from micropores in the stent body or mixed or bound to a polymer coating applied on stent, to inhibit neointimal tissue proliferation and thereby prevent restenosis.

[0002] The invention according to claim 1 also facilitates the performance of the stent in inhibiting restenosis.

[0003] Re-narrowing (restenosis) of an artherosclerotic coronary artery after percutaneous transluminal coronary angioplasty (PTCA) occurs in 10-50% of patients undergoing this procedure and subsequently requires either further angioplasty or coronary artery bypass graft. While the exact hormonal and cellular processes promoting restenosis are still being determined, our present understanding is that the process of PTCA, besides opening the artherosclerotically obstructed artery, also injures resident coronary arterial smooth muscle cells (SMC). In response to this injury, adhering platelets, infiltrating macrophages, leukocytes, or the smooth muscle cells (SMC) themselves release cell derived growth factors with subsequent proliferation and migration of medial SMC through the internal elastic lamina to the area of the vessel intima. Further proliferation and hyperplasia of intimal SMC and, most significantly, production of large amounts of extracellular matrix over a period of 3-6 months results in the filling in and narrowing of the vascular space sufficient to significantly obstruct coronary blood flow.

[0004] Several recent experimental approaches to preventing SMC proliferation have shown promise althrough the mechanisms for most agents employed are still unclear. Heparin is the best known and characterized agent causing inhibition of SMC proliferation both *in vitro* and in animal models of balloon angioplasty-mediated injury. The mechanism of SMC inhibition with heparin is still not known but may be due to any or all of the following: 1) reduced expression of the growth regulatory protooncogenes c-fos and c-myc, 2) reduced cellular production of tissue plasminogen activator; are 3) binding and dequestration of growth regulatory factors such as fibrovalent growth factor (FGF).

[0005] Other agents which have demonstrated the ability to reduce myointimal thickening in animal models of balloon vascular injury are angiopeptin (a somatostatin analog), calcium channel blockers, angiotensin converting enzyme inhibitors (captopril, cilazapril), cyclosporin A, trapidil (an antianginal, antiplatelet agent), terbinafine (antifungal), colchicine and taxol (antitubulin antiproliferatives), and *c-myc* and *c-myb* antinsense oligonucleotides.

[0006] Additionally, a goat antibody to the SMC mitogen platelet derived growth factor (PDGF) has been shown to be effective in reducing myointimal thickening in a rat model of balloon angioplasty injury, thereby implicating PDGF directly in the etiology of restenosis. Thus, while no therapy has as yet proven successful clinically in preventing restenosis after angioplasty, the *in vivo* experimental success of several agents known to inhibit SMC growth suggests that these agents as a class have the capacity to prevent clinical restenosis and deserve careful evaluation in humans.

[0007] Coronary heart disease is the major cause of death in men over the age of 40 and in women over the age of fifty in the western world. Most coronary artery-related deaths are due to atherosclerosis. Atherosclerotic lesions which limit or obstruct coronary blood flow are the major cause of ischemic heart disease related mortality and result in 500,000-600,000 deaths in the United States annually. To arrest the disease process and prevent the more advanced disease states in which the cardiac muscle itself is compromised, direct intervention has been employed via percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass graft (CABG).

[0008] PTCA is a procedure in which a small balloon-tipped catheter is passed down a narrowed coronary artery and then expanded to re-open the artery. It is currently performed in approximately 250,000-300,000 patients each year. The major advantage of this therapy is that patients in which the procedure is successful need not undergo the more invasive surgical procedure of coronary artery bypass graft. A major difficulty with PTCA is the problem of post-angioplasty closure of the vessel, both immediately after PTCA (acute reocclusion) and in the long term (restenosis).

[0009] The mechanism of acute reocclusion appears to involve several factors and may result from vascular recoil with resultant closure of the artery and/or deposition of blood platelets along the damaged length of the newly opened blood vessel followed by formation of a fibrin/red blood cell thrombus. Recently, intravascular stents have been examined as a means of preventing acute reclosure after PTCA.

[0010] Restenosis (chronic reclosure) after angioplasty is a more gradual process than acute reocclusion: 30% of patients with subtotal lesions and 50% of patients with chronic total lesions will go on to restenosis after angioplasty. While the exact mechanism for restenosis is still under active investigation, the general aspects of the restenosis process have been identified:

[0011] In the normal arterial wall, smooth muscle cells (SMC) proliferate at a low rate (<0.1%/day; ref). SMC in vessel wall exists in a 'contractile' phenotype characterized by 80-90% of the cell cytoplasmic volume occupied with the contractile apparatus. Endoplasmic reticulum, golgi bodies, and free ribosomes are few and located in the perinuclear region. Extracellular matrix surrounds SMC and is rich in heparin-like glycosylaminoglycans which are believed to be responsible for maintaining SMC in the contractile phenotypic state.

[0012] Upon pressure expansion of an intracoronary balloon catheter during angioplasty, smooth muscle cells within the arterial wall become injured. Cell derived

growth factors such as platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), etc. released from platelets (i. e., PDGF) adhering to the damaged arterial luminal surface, invading macrophages and/or leukocytes, or directly from SMC (i.e., BFGF) provoke a proliferation and migratory response in medial SMC. These cells undergo a phenotypic change from the contractile phenotyope to a 'synthetic' phenotype characterized by only few contractile filament bundles but extensive rough endoplasmic reticulum, golgi and free ribosomes. Proliferation/migration usually begins within 1-2 days post-injury and peaks at 2 days in the media, rapidly declining thereafter (Campbell et al., In: *Vascular Smooth Muscle Cells in Culture,* Campbell, J.H. and Campbell, G.R., Eds, CRC Press, Boca Ration, 1987, pp. 39-55); Clowes, A.W. and Schwartz,. S.M., Circ. Res. 56:139-145, 1985).

[0013] Finally, daughter synthetic cells migrate to the intimal layer of arterial smooth muscle and continue to proliferate. Proliferation and migration continues until the damaged luminal endothelial layer regenerates at which time proliferation ceases within the intima, usually within 7-14 days postinjury. The remaining increase in intimal thickening which occurs over the next 3-6 months is due to an increase in extracellular matrix rather than cell number. Thus, SMC migration and proliferation is an acute response to vessel injury while intimal hyperplasia is a more chronic response. (Liu et al., Circulation, 79:1374-1387, 1989).

[0014] Patients with symptomatic reocclusion require either repeat PTCA or CABG. Because 30-50% of patients undergoing PTCA will experience restenosis, restenosis has clearly limited the success of PTCA as a therapeutic approach to coronary artery disease. Because SMC proliferation and migration are intimately involved with the pathophysiological response to arterial injury, prevention of SMC proliferation and migration represents a target for pharmacological intervention in the prevention of restenosis.

Summary of the Invention:

Novel Features and Applications to Stent Technology

[0015] Currently, attempts to improve the clinical performance of stents have involved some variation of either applying a coating to the metal, attaching a covering or membrane, or embedding material on the surface via ion bombardment. A stent designed to include reservoirs according to claim 1 is a new approach which offers several important advantages over existing technologies.

Local Drug Delivery from a Stent to Inhibit Restenosis

[0016] In this application, it is desired to deliver a therapeutic agent to the site of arterial injury. The conventional approach has been to incorporate the therapeutic agent into a polymer material which is then coated on the stent. The ideal coating material must be able to adhere strongly to the metal stent both before and after expansion, be capable of retaining the drug at a sufficient load level to obtain the required dose, be able to release the drug in a controlled way over a period of several weeks, and be as thin as possible so as to minimize the increase in profile. In addition, the coating material should not contribute to any adverse response by the body (i.e., should be non-thrombogenic, non-inflammatory, etc.). To date, the ideal coating material has not been developed for this application.

[0017] An alternative would be to design the stent to contain reservoirs which could be loaded with the drug. A coating or membrane of biocompatable material could be applied over the reservoirs which would control the diffusion of the drug from the reservoirs to the artery wall.

[0018] One advantage of this system is that the properties of the coating can be optimized for achieving superior biocompatibility and adhesion properties, without the addition requirement of being able to load and release the drug. The size, shape, position, and number of reservoirs can be used to control the amount of drug, and therefore the dose delivered.

Description of the Drawings:

[0019] The invention will be better understood in connection with the following figures in which Figures 1 and 1A are top views and section views of a stent containing reservoirs as described in the present invention;

Figures 2a and 2b are similar views of an alternate embodiment of the stent with open ends;

Figures 3a and 3b are further alternate figures of a device containing a grooved reservoir; and

Figure 4 is a layout view of a device containing a reservoir as in Figure 3.

Detailed Description of the Invention

[0020] Pharmacological attempts to prevent restenosis by pharmacologic means have thus far been unsuccessful and all involve systemic administration of the trial agents. Neither aspirin-dipyridamole, ticlopidine, acute heparin administration, chronic warfarin (6 months) nor methylprednisolone have been effective in preventing restenosis although platelet inhibitors have been effective in preventing acute reocclusion after angioplasty. The calcium antagonists have also been unsuccessful in preventing restenosis, although they are still under study. Other agents currently under study include thromboxane inhibitors, prostacyclin mimetics,

platelet membrane receptor blockers, thrombin inhibitors and angiotensin converting enzyme inhibitors. These agents must be given systemically, however, and attainment of a therapeutically effective dose may not be possible; antiproliferative (or anti-restenosis) concentrations may exceed the known toxic concentrations of these agents so that levels sufficient to produce smooth muscle inhibition may not be reached (Lang et al., 42 Ann. Rev. Med., 127-132 (1991); Popma et al., 84 Circulation, .1426-1436 (1991)).

[0021] Additional clinical trials in which the effectiveness for preventing restenosis of dietary fish oil supplements, thromboxane receptor antagonists, cholesterol lowering agents, and serotonin antagonists has been examined have shown either conflicting or negative results so that no pharmacological agents are as yet clinically available to prevent post-angioplasty restenosis (Franklin, S.M. and Faxon, D.P., 4 Coronary Artery Disease, 232-242 (1993); Serruys, P.W. et al., 88 Circulation, (part 1) 1588-1601, (1993).

[0022] Conversely, stents have proven useful in preventing reducing the proliferation of restenosis. Stents, such as the stent 10 seen in layout in Figure 4, balloon-expandable slotted metal tubes (usually but not limited to stainless steel), which when expanded within the lumen of an angioplastied coronary artery, provide structural support to the arterial wall. This support is helpful in maintaining an open path for blood flow. In two randomized clinical trials, stents were shown to increase angiographic success after PTCA, increase the stenosed blood vessel lumen and to reduce the lesion recurrence at 6 months (Serruys et al., 331 New Eng Jour. Med, 495, (1994); Fischman et al., 331 New Eng Jour. Med, 496-501 (1994). Additionally, in a preliminary trial, heparin coated stents appear to possess the same benefit of reduction in stenosis diameter at follow-up as was observed with non-heparin coated stents. Additionally, heparin coating appears to have the added benefit of producing a reduction in sub-acute thrombosis after stent implantation (Serruys et al., 93 Circulation, 412-422, (1996). Thus, 1) sustained mechanical expansion of a stenosed coronary artery has been shown to provide some measure of restenosis prevention, and 2) coating of stents with heparin has demonstrated both the feasibility and the clinical usefulness of delivering drugs to local, injured tissue off the surface of the stent.

[0023] Numerous agents are being actively studied as antiproliferative agents for use in restenosis and have shown some activity in experimental animal models. These include: heparin and heparin fragments (Clowes and Kamovsky, 265 Nature, 25-626, (1977); Guyton, J. R. et al. 46 Circ. Res., 625-634, (1980); Clowes, A.W. and Clowes, M.M., 52 Lab. Invest., 611-616, (1985); Clowes, A.W. and Clowes, M.M., 58 Circ. Res., 839-845 (1986); Majesky et al., 61 Circ Res., 296-300, (1987); Snow et al., 137 Am. J. Pathol., 313-330 (1990); Okada, T. et al., 25 Neurosurgery, 92-898, (1989) colchicine (Currier, J.W. et al., 80 Circulation, 11-66, (1989), taxol (ref), agiotensin converting enzyme (ACE) inhibitors (Powell, J.S. et al., 245 Science, 186-188 (1989), angiopeptin (Lundergan, C.F. et al., 17 Am. J. Cardiol. (Suppl. B); 132B-136B (1991), Cyclosporin A (Jonasson, L. et. al., 85 Proc. Nati, Acad. Sci., 2303 (1988), goat-anti-rabbit PDGF antibody (Ferns, G.A.A., et al., 253 Science, 1129-1132 (1991), terbinafine (Nemecek, G.M. et al., 248 J. Pharmacol. Exp. Thera., 1167-11747 (1989), trapidil (Liu, M.W. et al., 81 Circulation, 1089-1093 (1990), interferon-gamma (Hansson, G.K. and Holm, 84 J. Circulation, 1266-1272 (1991), steroids (Colburn, M. D. et al., 15 J. Vasc. Surg., 510-518 (1992), see also Berk, B.C. et al., 17 J. Am. Coll. Cardiol., 111B-1 17B (1991), ionizing radiation (ref), fusion toxins (ref) anti-sense oligonucleotides (ref), gene vectors (ref), and rapamycin (see below).

[0024] Of particular interest is rapamycin. Rapamycin is a macrolide antibiotic which blocks IL-2- mediated T-cell proliferation and possesses antiinflammatory activity. While the precise mechanism of rapamycin is still under active investigation, rapamycin has been shown to prevent the G_1 to S phase progression of T-cells through the cell cycle by inhibiting specific cell cyclins and cyclin-dependent protein kinases (Siekierka, Immunol. Res. 13: 110-116, 1994). The antiproliferative action of rapamycin is not limited to T-cells; Marx et al. (Circ Res 76:412-417, 1995) have demonstrated that rapamycin prevents proliferation of both rat and human SMC in vitro while Poon et al. have shown the rat, porcine, and human SMC migratin can also be inhibited by rapamycin (J Clin Invest 98: 2277-2283, 1996). Thus, rapamycin is capable of inhibiting both the inflammatory response known to occur after arterial injury and stent implantation, as well as the SMC hyperproliferative response. In fact, the combined effects of rapamycin have been demonstrated to result in a diminished SMC hyperproliferative response in a rat femoral artery graft model and in both rat and porcine arterial balloon injury models (Gregory et al., Transplantation 55:1409-1418, 1993; Gallo et al., in press, (1997)). These observations clearly support the potential use of rapamycin in the clinical setting of post-angioplasty restenosis.

[0025] Although the ideal agent for restenosis has not yet been identified, some desired properties are clear: inhibition of local thrombosis without the risk systemic bleeding complications and continuous and prevention of the dequale of arterial injury, including local inflammation and sustained prevention smooth muscle proliferation at the site of angioplasty without serious systemic complications. Inasmuch as stents prevent at least a portion of the restenosis process, an agent which prevents inflammation and the proliferation of SMC combined with a stent may provide the most efficacious treatment for post-angioplasty restenosis.

Experiments

[0026] Agents: Rapamycin (sirolimus) structural ana-

logs (macrocyclic lactones) and inhibitors of cell-cycle progression.

Delivery Methods:

[0027] These can vary:

- Local delivery of such agents (rapamycin) from the struts of a stent, from a stent graft, grafts, stent cover or sheath.
- Involving comixture with polymers (both degradable and nondegrading) to hold the drug to the stent or graft.
- or entrapping the drug into the metal of the stent or graft body which has been modified to contain micropores or channels, as will be explained further herein.
- or including covalent binding of the drug to the stent via solution chemistry techniques (such as via the Carmeda process) or dry chemistry techniques (e. g. vapour deposition methods such as rf-plasma polymerization) and combinations thereof.
- Catheter delivery intravascularly from a tandem balloon or a porous balloon for intramural uptake
- Extravascular delivery by the pericardial route
- Extravascular delivery by the advential application of sustained release formulations.

[0028] Uses: for inhibition of cell proliferation to prevent neointimal proliferation and restenosis.

prevention of tumor expansion from stents
prevent ingrowth of tissue into catheters and shunts inducing their failure.

1. Experimental Stent Delivery Method - Delivery from Polymer Matrix:

[0029] Solution of Rapamycin, prepared in a solvent miscible with polymer carrier solution, is mixed with solution of polymer at final concentration range 0.001 weight % to 30 weight % of drug. Polymers are biocompatible (i.e., not elicit any negative tissue reaction or promote mural thrombus formation) and degradable, such as lactone-based polyesters or copolyesters, e.g., polylactide, polycaprolacton-glycolide,polyorthoesters, polyanhydrides; poly-aminoacids; polysaccharides; polyphosphazenes; poly(ether-ester) copolymers, e.g., PEO-PLLA, or blends thereof. Nonabsorbable biocompatible polymers are also suitable candidates. Polymers such as polydimethylsiolxane; poly(ethylene-vingylacetate); acrylate based polymers or copolymers, e.g., poly (hydroxyethyl methylmethacrylate, polyvinyl pyrrolidinone; fluorinated polymers such as polytetrafluoroethylene; cellulose esters.

[0030] Polymer/drug mixture is applied to the surfaces of the stent by either dip-coating, or spray coating, or brush coating or dip/spin coating or combinations thereof, and the solvent allowed to evaporate to leave a film with entrapped rapamycin.

2. Experimental Stent Delivery Method - Delivery from Microporous Depots in Stent Through a Polymer Membrane Coating:

[0031] Stent, whose body has been modified to contain micropores or channels is dipped into a solution of Rapamycin, range 0.001 wt% to saturated, in organic solvent such as acetone or methylene chloride, for sufficient time to allow solution to permeate into the pores. (The dipping solution can also be compressed to improve the loading efficiency.) After solvent has been allowed to evaporate, the stent is dipped briefly in fresh solvent to remove excess surface bound drug. A solution of polymer, chosen from any identified in the first experimental method, is applied to the stent as detailed above. This outerlayer of polymer will act as diffusion-controller for release of drug.

3. Experimental Stent Delivery Method - Delivery via lysis of a Covalent Drug Tether

[0032] Rapamycin is modified to contain a hydrolytically or enzymatically labile covalent bond for attaching to the surface of the stent which itself has been chemically derivatized to allow covalent immobilization. Covalent bonds such as ester, amides or anhydrides may be suitable for this.

4. Experimental Method - Pericardial Delivery

[0033] A: Polymeric Sheet Rapamycin is combined at concentration range previously highlighted, with a degradable polymer such as poly(caprolactone-gylcolide) or non-degradable polymer, e.g., polydimethylsiloxane, and mixture cast as a thin sheet, thickness range 10μ to 1000μ. The resulting sheet can be wrapped perivascularly on the target vessel. Preference would be for the absorbable polymer.

[0034] B: Conformal Coating: Rapamycin is combined with a polymer that has a melting temperature just above 37°C, range 40°-45°C. Mixture is applied in a molten state to the external side of the target vessel. Upon cooling to body temperature the mixture solidifies conformally to the vessel wall. Both non-degradable and absorbable biocompatible polymers are suitable.

[0035] As seen in the figures it is also possible to modify currently manufactured stents in order to adequately provide the drug dosages such as rapamycin. As seen in Figures 1a, 2a and 3a, any stent strut 10, 20, 30 can

be modified to have a certain reservoir or channel 11, 21, 31. Each of these reservoirs can be open or closed as desired. These reservoirs can hold the drug to be delivered. Figure 4 shows a stent 40 with a reservoir 45 created at the apex of a flexible strut. Of course, this reservoir 45 is intended to be useful to deliver rapamycin or any other drug at a specific point of flexibility of the stent. Accordingly, this concept can be useful for "second generation" type stents.

[0036] In any of the foregoing devices, however, it is useful to have the drug dosage applied with enough specificity and enough concentration to provide an effective dosage in the lesion area. In this regard, the reservoir size in the stent struts must be kept at a size of about $1.27 \cdot 10^{-5}$ m 0.0005") to about $7.62 \cdot 10^{-5}$ m 0.003"). Then, it should be possible to adequately apply the drug dosage at the desired location and in the desired amount.

[0037] These and other concepts will are disclosed herein. It would be apparent to the reader that modifications are possible to the stent or the drug dosage applied. In any event, however, the any obvious modifications should be perceived to fall within the scope of the invention which is to be realized from the attached claims and their equivalents.

Claims

1. A stent comprising:

   a generally thin walled cylinder, said cylinder containing a plurality of struts (10, 20, 30), said struts expandable dependent on the amount of force applied to said strut, and said struts having a generally uniform thickness; and a channel (11, 21, 31) formed in at least one of said struts, said channel having a closed perimeter on three sides and an open top, and said channel smaller in all dimensions than said strut, said channel containing a therapeutic agent applied therein.

2. A stent according to claim 1 wherein said channel has a generally rectangular perimeter.

3. A stent according to claim 2 wherein said therapeutic agent is rapamycin coated to said channel.

4. The stent of claim 3 wherein said channel is rectangular in shape.

5. The stent of claim 3 containing struts with said channels.

6. The stent of claim 3 wherein said channel is laser cut into said strut.

7. The stent of claim 1 wherein the therapeutic agent is rapamycin.

8. A stent comprising a generally thin walled structure containing a plurality of struts (10, 20, 30), the struts expandable to assume the shape of a lumen into which the stent is emplaced, said struts having a thickness, and a channel (11, 21, 31) formed in at least one of said struts, said channel having a closed perimeter on three sides and an open top, and said channel smaller in all dimensions than said strut, said channel containing a therapeutic agent applied therein.

Patentansprüche

1. Stent umfassend:

   einen im Allgemeinen dünnwandigen Zylinder, wobei der Zylinder eine Vielzahl von Verstrebungen (10, 20, 30)-enthält, wobei die expandierbaren Verstrebungen von dem Ausmaß an Kraft abhängen, die auf die Verstrebung ausgeübt wird, und die Verstrebungen eine im Allgemeinen gleichförmige Dikke aufweisen; und einen Kanal (11, 21, 31), der in wenigstens einer der Verstrebungen gebildet ist, wobei der Kanal auf drei Seiten eine geschlossene äußere Begrenzung und eine offene Oberseite aufweist und der Kanal in allen Dimensionen kleiner ist als die Verstrebung und der Kanal ein darin aufgetragenes therapeutisches Agens enthält.

2. Stent nach Anspruch 1, wobei der Kanal eine im Wesentlichen rechteckige äußere Begrenzung aufweist.

3. Stent nach Anspruch 2, wobei das therapeutische Agens auf den Kanal beschichtetes Rapamycin ist.

4. Stent nach Anspruch 3, wobei der Kanal eine rechtwinklige Form aufweist.

5. Stent nach Anspruch 3 enthaltend Verstrebungen mit den Kanälen.

6. Stent nach Anspruch 3, wobei der Kanal mittels eines Lasers in die Verstrebung geschnitten ist.

7. Stent nach Anspruch 1, wobei das therapeutische Agens Rapamycin ist.

8. Stent umfassend eine im Allgemeinen dünnwandige Struktur mit einer Vielzahl von Verstrebungen (10, 20, 30), wobei die Verstrebungen expandierbar sind, um die Form eines Lumens anzunehmen, in

die der Stent eingesetzt wird, die Verstrebungen eine Dicke aufweisen und ein Kanal (11, 21, 31) in wenigstens einer der Verstrebungen gebildet ist, der Kanal eine geschlossene äußere Begrenzung auf drei Seiten und eine offene Oberseite aufweist, und der Kanal in allen Dimensionen kleiner ist als die Verstrebung und der Kanal ein darin aufgetragenes therapeutisches Agens enthält.

Revendications

1. Support interne comprenant :

   - un cylindre généralement à paroi mince, ledit cylindre contenant une pluralité de pattes (10, 20, 30), lesdites pattes étant susceptibles de s'étendre suivant l'intensité de la force appliquée à ladite patte et lesdites pattes ayant une épaisseur généralement uniforme, un canal (11, 21, 31) étant ménagé à l'intérieur d'au moins une desdites pattes, ledit canal ayant un périmètre fermé sur trois côtés et une partie supérieure ouverte, ledit canal étant plus petit, dans toutes les dimensions, que ladite patte et ledit canal contenant un agent thérapeutique appliqué à l'intérieur de celui-ci.

2. Support interne selon la revendication 1, dans lequel ledit canal a un périmètre généralement rectangulaire.

3. Support interne selon la revendication 2, dans lequel ledit agent thérapeutique est de la rapamycine dont est revêtu ledit canal.

4. Support interne selon la revendication 3, dans lequel ledit canal est de forme rectangulaire.

5. Support interne selon la revendication 3, contenant des pattes avec lesdits canaux.

6. Support interne selon la revendication 3, dans lequel ledit canal est découpé au laser à l'intérieur de ladite patte.

7. Support interne selon la revendication 1, dans lequel l'agent thérapeutique est de la rapamycine.

8. Support interne comprenant une structure généralement à paroi mince contenant une pluralité de pattes (10, 20, 30), lesdites pattes étant susceptibles de s'étendre pour prendre la forme d'une lumière dans laquelle est placé le support interne, lesdites pattes ayant une épaisseur, un canal (11, 21, 31) étant ménagé à l'intérieur d'au moins une desdites pattes, ledit canal ayant un périmètre fermé sur trois côtés et une partie supérieure ouverte, ledit canal étant plus petit, dans toutes les dimensions, que ladite patte et ledit canal contenant un agent thérapeutique appliqué à l'intérieur de celui-ci.

FIG. 1

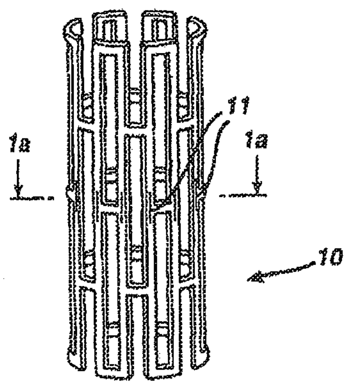


FIG. 1a

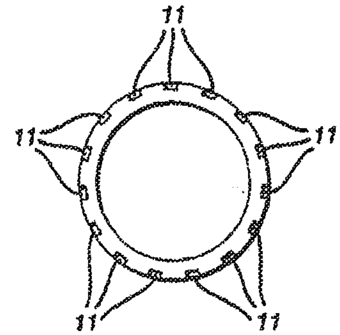


FIG. 2a

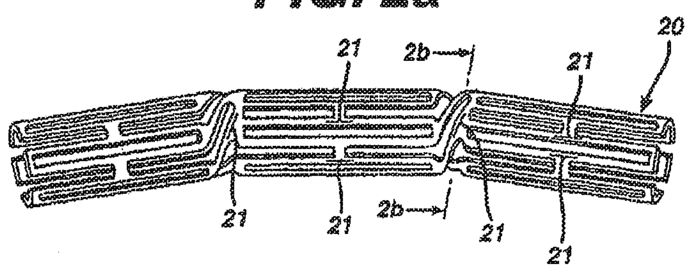


FIG. 2b

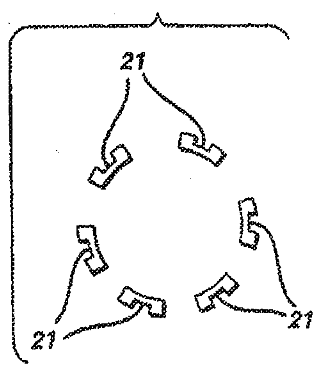


FIG. 3a

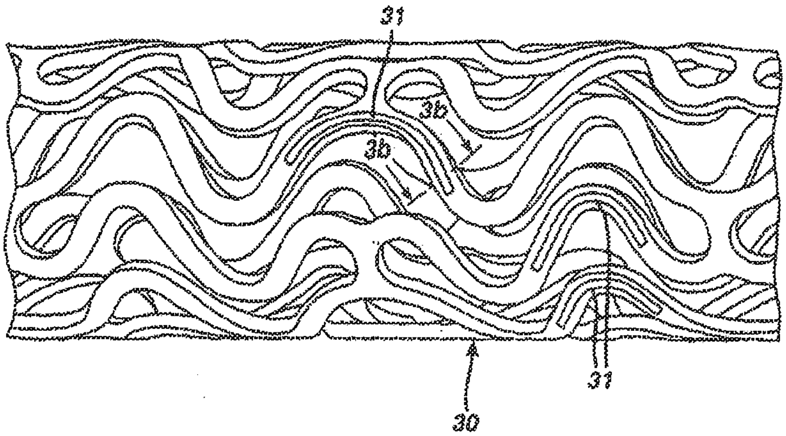


FIG. 3b

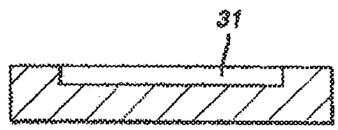
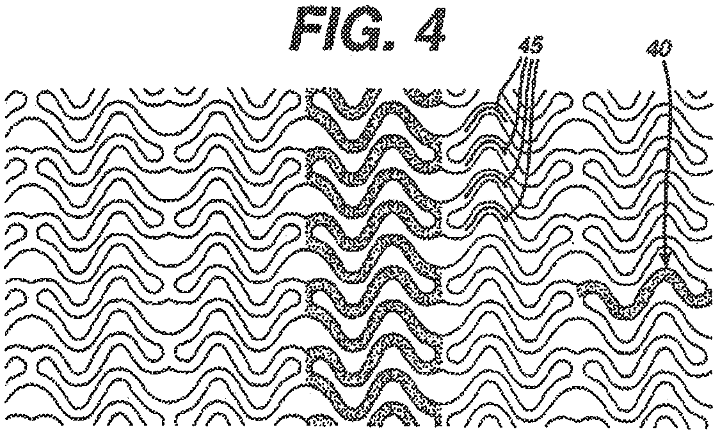


FIG. 4



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(54) **Process for coating stents**

(57) A process is provided for coating stents having a first and second surface with passages there between to avoid blockage and bridging of the passages. The process comprises contacting the stent with a liquid coating solution containing a film forming biocompatible polymer under conditions suitable to allow the film forming biocompatible polymer to coat at least one surface of the stent while maintaining a fluid flow through said passages sufficient prevent the film forming biocompatible polymer from substantially blocking said passages. Also described are stents coated by this process.

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Description

Field of the Invention

[0001] This application claims benefit from U.S. Provisional Application No. 60/91,217 filed June 30, 1998, which is hereby incorporated by reference herein. The invention relates generally to a process for coating surgical devices. More specifically this invention relates to an improved process for coating stents and the like.

Background of the Invention

[0002] Stents, which are generally open tubular structures, have become increasingly important in medical procedures to restore the function of body lumens. Stents are now commonly used in translumenial procedures such as angioplasty to restore an adequate blood flow to the heart. However, stents may stimulate foreign body reactions that result in thrombosis or restenosis. To avoid these complications a variety of stent coatings and compositions have been proposed in the literature both to reduce the incidence of these complications or other complications and restore tissue function by itself or by delivering therapeutic compound to the lumen.

[0003] Stents generally are coated by simple dip or spray coating of the stent with polymer or polymer and a pharmaceutical/therapeutic agent or drug. These methods are acceptable for early stent designs that were of open construction fabricated from wires (Wiktor stent) or from ribbons (Gianturco). Dip coating with relatively low coating weights (about 4% polymer) could successfully coat such stents without any problems such as excess coating bridging (i.e. forming a film across) the open space between structural members of the device. This bridging is of particular concern when coating more modern stents that are of less open construction, such as the Palmaz-Schatz, Crown, Multilink or GFX stents. Bridging of the open space (slots) is undesirable because it can interfere with the mechanical performance of the stent, such as expansion during deployment in a vessel lumen. Bridges may rupture upon expansion and provide sites that activate platelet deposition by creating flow disturbances in the adjacent hemodynamic environment or pieces of the bridging film may break off and cause further complications. Bridging of the open slots may also prevent endothelial cell migration complicating the endothelial cell encapsulation of the stent.

[0004] Similarly, spray coating can be problematic in that there is a significant amount of spray lost during the process and many of the pharmaceutical agents that one would like to incorporate in the device are quite costly. In addition, in some cases it would be desirable to provide coated stents with high levels of coating and drug. High concentration coatings (-15% polymer with additional drug) are the preferred means to achieve high drug loading. Multiple dip-coating has been described in the literature as a means to build thicker coatings on the stent. However, composition and phase dispersion of the pharmaceutical agents affect sustained release. In addition, the application of multiple dip coats from low concentration solutions often has the effect of reaching a limiting loading level as an equilibrium is reached between the solution concentration and the amount of coating, with or without pharmaceutical agent, deposited on the stent.

Summary of the Invention

[0005] We have discovered a process for coating stents that avoids bridging and allows for preferential coating of stent surfaces. The process comprises contacting a stent having a first and second surface with passages there between with a liquid coating solution containing a film forming biocompatible polymer under conditions suitable to allow the film forming biocompatible polymer to coat at least one surface of the stent while maintaining a fluid flow through said passages sufficient to prevent the film forming biocompatible polymer from substantially blocking said passages.

[0006] In a preferred embodiment of the present invention the coating process would comprise placing a tubular stent having a first and second surface with passages there between on a mandrel and contacting the stent and mandrel with a liquid coating solution containing a film forming biocompatible polymer under conditions suitable to allow the film forming biocompatible polymer to coat at least one surface of the stent while moving the stent relative to the mandrel to cause fluid flow through said passages sufficient to prevent the film forming biocompatible polymer from substantially blocking said passages.

[0007] In another embodiment of the present invention there is provided a coated stent, comprising a tubular stent having a first and second surface with passages there between, coated with a film-forming biocompatible polymer wherein the polymer coating is greater than 0.5 percent by weight of the coated stent and the passages are not substantially blocked by the bridging of the polymer coating.

Brief Description of the Figures

[0008] Figure 1 illustrates a perspective view of a stent prior to coating.

[0009] Figure 2 is a perspective view that illustrates the placement of a stent on a mandrel prior to coating.

[0010] Figure 3 illustrates the movement of the stent relative to the mandrel in the after removal from the coating bath during the coating process.

[0011] Figure 4 is an enlarged view of a portion of the coated stent that illustrates the substantial absence of bridging of the stent slots or passages.

[0012] Figure 5 is a pictomicrograph that illustrates a stent that has been coated by conventional dip coating process with about a 4 weight percent coating solution.

[0013] Figure 6 is a pictomicrograph that illustrate a stent that has been coated by the inventive coating process with about a 13 weight percent coating solution.

[0014] Figure 7 is a graphical illustration of the in vitro release profile of a coated stent.

[0015] Figure 8 is a graphical illustration of the in vivo release profile of a coated stent.

Detailed Description

[0016] The present invention provides a process for coating medical devices. The process described herein is well suited to coating medical devices that have passages that may otherwise be blocked or have bridges formed by conventional dip coating. As previously discussed avoiding the formation of bridges is especially important in the coating of perforated structures such as stents. Bridging is a significant problem with stents with passages with a minor dimension less than about 125 mils, especially with passages having a minor dimension smaller than about 50 mils.

[0017] Stents are generally cylindrical and perforated with passages that are slots, ovoid, circular or the like shape. Stents may also be composed of helically wound or serpentine wire structures in which the spaces between the wires form the passages. Stents may be flat perforated structures that are subsequently rolled to form tubular structures or cylindrical structures that are woven, wrapped, drilled, etched or cut to form passages. Examples of stents that may be advantageously coated by the present process include but are not limited stents described in the following U.S. Patent Nos. 4,733,665 (hereinafter the Palmaz stent which is illustrated in Figure 1); 4,800,882 (hereinafter the Gianturco stent); 4,886,062 (hereinafter the Wiktor stent) and 5,514,154 (hereinafter the Guidant RX Multilink™ stent). These stents can be made of biocompatible materials including biostable and bioabsorbable materials. Suitable biocompatible metals include, but are not limited to, stainless steel, tantalum, titanium alloys (including nitinol), and cobalt alloys (including cobalt-chromium-nickel alloys). Suitable nonmetallic biocompatible materials include, but are not limited to, polyamides, polyolefins (i.e. polypropylene, polyethylene etc.), nonabsorbable polyesters (i.e. polyethylene terephthalate), and bioabsorbable aliphatic polyesters (i.e. homopolymers and copolymers of lactic acid, glycolic acid, lactide, glycolide, para-dioxanone, trimethylene carbonate, ε-caprolactone, etc. and blends thereof).

[0018] The present invention utilizes fluid flow or movement through the passages in the perforated medical device to avoid the formation of blockages or bridges. The fluid flow can be provided by active flow systems such as a perforated manifold inserted in the stent to circulate the coating fluid through the passages or can be created by placing the stent on a mandrel or in a small tube that is moved relative to the stent during the coating process to create sufficient fluid flow through the passages and thereby avoid the formation of blockages or bridges.

[0019] In one embodiment of the present invention as illustrated in Figure 2, a stent 2 is placed over a mandrel 6 that is smaller than the inner diameter d of the stent's intraluminal passage way 12 and dipped into the coating solution. The coated stent is moved relative to the mandrel after it is removed from the coating solution (preferably in one direction). Figure 3 illustrates the movement of the stent 2 relative to the mandrel 6 after it is removed from bath. The relative outer diameter of the mandrel and inner diameter of the stent are such that after dipping, while the coating is still wet, the movement of the stent along the mandrel's length clears the passages (slots) 10 which remain so on drying. The relative motion of the stent and mandrel, with limited clearance between the stent and mandrel, generates high shear rates which break the surface tension associated with the coating film filling the slots and provides smooth, defect free coating on the stent. Preferably the stent will be moved to an area of the mandrel that has not contacted the coating solution. As is illustrated in Figure 3 that provides a perspective view of the stent 2 after being coated with coating 14. There are additional advantages: the coatings can be of high concentration and by proper choice of the mandrel diameter to stent diameter (the clearance), the relative thickness of the inner and outer coating of the stent can be controlled. For example, the stent coating can be thicker on the outer surface to contact the luminal wall or thicker on the interior surface to interact with the fluid stream.

[0020] The mandrel may be of varying designs (i.e. tapered cones, cylindrical, slotted cylinders, mandrels having cross-sections that are ovoid, triangular or polygonal and would include shafts with veins or paddles). Additionally, the movement of the mandrel relative to the stent may not only be laterally, but may also consist of rotational movement. Object of the mandrel design being to assure sufficient shear flow relative to the passages to insure that the passages do not be come blocked.

[0021] Film-forming polymers that can be used for coatings in this application can be absorbable or non-absorbable and must be biocompatible to minimize irritation to the vessel wall. The polymer may be either biostable or bioabsorbable depending on the desired rate of release or the desired degree of polymer stability, but a bioabsorbable polymer

is preferred since, unlike biostable polymer, it will not be present long after implantation to cause any adverse, chronic local response. Furthermore, bioabsorbable polymers do not present the risk that over extended periods of time there could be an adhesion loss between the stent and coating caused by the stresses of the biological environment that could dislodge the coating and introduce further problems even after the stent is encapsulated in tissue.

[0022] Suitable film-forming bioabsorbable polymers that could be used include polymers selected from the group consisting of aliphatic polyesters, poly(amino acids), copoly(ether-esters), polyalkylenes oxalates, polyamides, poly (iminocarbonates), polyorthoesters, polyoxaesters, polyamidoesters, polyoxaesters containing amido groups, poly(anhydrides), polyphosphazenes, biomolecules and blends thereof. For the purpose of this invention aliphatic polyesters include homopolymers and copolymers of lactide (which includes lactic acid d-,l- and meso lactide), ε-caprolactone, glycolide (including glycolic acid), hydroxybutyrate, hydroxyvalerate, para-dioxanone, trimethylene carbonate (and its alkyl derivatives), 1,4-dioxepan-2-one, 1,5-dioxepan-2-one, 6,6-dimethyl-1,4-dioxan-2-one and polymer blends thereof. Poly(iminocarbonate) for the purpose of this invention include as described by Kemnitzer and Kohn, in the Handbook of Biodegradable Polymers, edited by Domb, Kost and Wisemen, Hardwood Academic Press, 1997, pages 251-272. Copoly(ether-esters) for the purpose of this invention include those copolyester-ethers described in Journal of Biomaterials Research, Vol. 22, pages 993-1009, 1988 by Cohn and Younes and Cohn, Polymer Preprints (ACS Division of Polymer Chemistry) Vol. 30(1), page 498, 1989 (e.g. PEO/PLA). Polyalkylene oxalates for the purpose of this invention include Patent Nos. 4,208,511; 4,141,087; 4,130,639; 4,140,678; 4,105,034; and 4,205,399 (incorporated by reference herein) . Polyphosphazenes, co-, ter- and higher order mixed monomer based polymers made from L-lactide, D,L-lactide, lactic acid, glycolide, glycolic acid, para-dioxanone, trimethylene carbonate and ε-caprolactone such as are described by Allcock in The Encyclopedia of Polymer Science, Vol. 13, pages 31-41, Wiley Intersciences, John Wiley & Sons, 1988 and by Vandorpe, Schacht, Dejardin and Lemmouchi in the Handbook of Biodegradable Polymers, edited by Domb, Kost and Wisemen, Hardwood Academic Press, 1997, pages 161-182 (which are hereby incorporated by reference herein). Polyanhydrides from diacids of the form $HOOC-C_6H_4-O-(CH_2)_m-O-C_6H_4-COOH$ where m is an integer in the range of from 2 to 8 and copolymers thereof with aliphatic alpha-omega diacids of up to 12 carbons. Polyoxaesters polyoxaamides and polyoxaesters containing amines and/or amido groups are described in one or more of the following U.S. Patent Nos. 5,464,929; 5,595,751; 5,597,579; 5,607,687; 5,618,552; 5,620,698; 5,645,850; 5,648,088; 5,698,213 and 5,700,583; (which are incorporated herein by reference). Polyorthoesters such as those described by Heller in Handbook of Biodegradable Polymers, edited by Domb, Kost and Wisemen, Hardwood Academic Press, 1997, pages 99-118 (hereby incorporated herein by reference). Film-forming polymeric biomolecules for the purpose of this invention include naturally occurring materials that may be enzymatically degraded in the human body or are hydrolytically unstable in the human body such as fibrin, fibrinogen, collagen, elastin, and absorbable biocompatable polysaccharides such as chitosan, starch, fatty acids (and esters thereof), glucoso-glycans and hyaluronic acid.

[0023] Suitable film-forming biostable polymers with relatively low chronic tissue response, such as polyurethanes, silicones, poly(meth)acrylates, polyesters, polyalkyl oxides (polyethylene oxide), polyvinyl alcohols, polyethylene glycols and polyvinyl pyrrolidone, as well as, hydrogels such as those formed from crosslinked polyvinyl pyrrolidinone and polyesters could also be used. Other polymers could also be used if they can be dissolved, cured or polymerized on the stent. These include polyolefins, polyisobutylene and ethylene-alphaolefin copolymers; acrylic polymers (including methacrylate) and copolymers, vinyl halide polymers and copolymers, such as polyvinyl chloride; polyvinyl ethers, such as polyvinyl methyl ether; polyvinylidene halides such as polyvinylidene fluoride and polyvinylidene chloride; polyacrylonitrile, polyvinyl ketones; polyvinyl aromatics such as polystyrene; polyvinyl esters such as polyvinyl acetate; copolymers of vinyl monomers with each other and olefins, such as etheylene- methyl methacrylate copolymers, acrylonitrile-styrene copolymers, ABS resins and ethylene-vinyl acetate copolymers; polyamides,such as Nylon 66 and polycaprolactam; alkyd resins; polycarbonates; polyoxymethylenes; polyimides; polyethers; epoxy resins, polyurethanes; rayon; rayon-triacetate, cellulose, cellulose acetate, cellulose acetate butyrate; cellophane; cellulose nitrate; cellulose propionate; cellulose ethers (i.e. carboxymethyl cellulose and hydoxyalkyl celluloses); and combinations thereof. Polyamides for the purpose of this application would also include polyamides of the form-$NH-(CH_2)_n-CO-$ and $NH-(CH_2)_x-NH-CO-(CH_2)_y-CO$, wherein n is preferably an integer in from 6 to 13; x is an integer in the range of form 6 to 12; and y is an integer in the range of from 4 to 16. The list provided above is illustrative but not limiting.

[0024] The polymers used for coatings must be film-forming polymers that have molecular weight high enough as to not be waxy or tacky. The polymers also must adhere to the stent and not be so readily deformable after deposition on the stent as to be able to be displaced by hemodynamic stresses. The polymers molecular weight be high enough to provide sufficient toughness so that the polymers will not to be rubbed off during handling or deployment of the stent and must not crack during expansion of the stent. The melting point of the polymer used in the present invention should have a melting temperature above 40°C, preferably above about 45°C, more preferably above 50°C and most preferably above 55°C.

[0025] The preferable coatings to use for this application are bioabsorbable elastomers, more preferably aliphatic polyester elastomers. In the proper proportions aliphatic polyester copolymers are elastomers. Elastomers present the advantage that they tend to adhere well to the metal stents and can withstand significant deformation without cracking.

The high elongation and good adhesion provide superior performance to other polymer coatings when the coated stent is expanded. Examples of suitable bioabsorbable elastomers are described in U.S. Patent No. 5,468,253 hereby incorporated by reference. Preferably the bioabsorbable biocompatible elastomers based on aliphatic polyester, including but not limited to those selected from the group consisting of elastomeric copolymers of ε-caprolactone and glycolide (preferably having a mole ratio of ε-caprolactone to glycolide of from about 35:65 to about 65:35, more preferably 45:55 to 35:65) elastomeric copolymers of E-caprolactone and lactide, including L-lactide, D-lactide blends thereof or lactic acid copolymers (preferably having a mole ratio of ε-caprolactone to lactide of from about 35:65 to about 90:10 and more preferably from about 35:65 to about 65:35 and most preferably from about 45:55 to 30:70 or from about 90:10 to about 80:20) elastomeric copolymers of p-dioxanone (1,4-dioxan-2-one) and lactide including L-lactide, D-lactide and lactic acid (preferably having a mole ratio of p-dioxanone to lactide of from about 40:60 to about 60:40) elastomeric copolymers of ε-caprolactone and p-dioxanone (preferably having a mole ratio of ε-caprolactone to p-dioxanone of from about 30:70 to about 70:30) elastomeric copolymers of p-dioxanone and trimethylene carbonate (preferably having a mole ratio of p-dioxanone to trimethylene carbonate of from about 30:70 to about 70:30), elastomeric copolymers of trimethylene carbonate and glycolide (preferably having a mole ratio of trimethylene carbonate to glycolide of from about 30:70 to about 70:30), elastomeric copolymer of trimethylene carbonate and lactide including L-lactide, D-lactide, blends thereof or lactic acid copolymers (preferably having a mole ratio of trimethylene carbonate to lactide of from about 30:70 to about 70:30) and blends thereof. As is well known in the art these aliphatic polyester copolymers have different hydrolysis rates, therefore, the choice of elastomer may in part be based on the requirements for the coatings adsorption. For example ε-caprolactone-co-glycolide copolymer (45:55 mole percent, respectively) films lose 90% of their initial strength after 2 weeks in simulated physiological buffer whereas the ε-caprolactone-co-lactide copolymers (40:60 mole percent, respectively) loses all of its strength between 12 and 16 weeks in the same buffer. Mixtures of the fast hydrolyzing and slow hydrolyzing polymers can be used to adjust the time of strength retention.

[0026] The preferred bioabsorbable elastomeric polymers should have an inherent viscosity of from about 1.0 dL/g to about 4 dL/g, preferably an inherent viscosity of from about 1.0 dL/g to about 2 dL/g and most preferably an inherent viscosity of from about 1.2 dL/g to about 2 dL/g as determined at 25°C in a 0.1 gram per deciliter (g/dL) solution of polymer in hexafluoroisopropanol (HFIP).

[0027] The solvent is chosen such that there is the proper balance of viscosity, deposition level of the polymer, solubility of the pharmaceutical agent, wetting of the stent and evaporation rate of the solvent to properly coat the stents. In the preferred embodiment, the solvent is chosen such the pharmaceutical agent and the polymer are both soluble in the solvent. In some cases, the solvent must be chosen such that the coating polymer is soluble in the solvent and such that pharmaceutical agent is dispersed in the polymer solution in the solvent. In that case the solvent chosen must be able to suspend small particles of the pharmaceutical agent without causing them to aggregate or agglomerate into collections of particles that would clog the slots of the stent when applied. Although the goal is to dry the solvent completely from the coating during processing, it is a great advantage for the solvent to be non-toxic, non-carcinogenic and environmentally benign. Mixed solvent systems can also be used to control viscosity and evaporation rates. In all cases, the solvent must not react with or inactivate the pharmaceutical agent or react with the coating polymer. Preferred solvents include by are not limited to: acetone, N-methylpyrrolidone (NMP), dimethyl sulfoxide (DMSO), toluene, methylene chloride, chloroform, 1,1,2-trichloroethane (TCE), various freons, dioxane, ethyl acetate, tetrahydrofuran (THF), dimethylformamide (DMF), and dimethylacetamide (DMAC).

[0028] The film-forming biocompatible polymer coatings are generally applied to reduce local turbulence in blood flow through the stent, as well as, adverse tissue reactions. The coating may also be used to administer a pharmaceutically active material to the site of the stents placement. Generally, the amount of polymer coating to be placed on the stent will vary with the polymer and the stent design and the desired effect of the coating. As a guideline the amount of coating may range from about 0.5 to about 20 as a percent of the total weight of the stent after coating and preferably will range from about 1 to about 15 percent. The polymer coatings may be applied in one or more coating steps depending on the amount of polymer to be applied. Different polymers may also be used for different layers in the stent coating. In fact it is highly advantageous to use a dilute first coating solution as primer to promote adhesion of a subsequent coating layers that may contain pharmaceutically active materials.

[0029] Additionally, a top coating can be applied to delay release of the pharmaceutical agent, or they could be used as the matrix for the delivery of a different pharmaceutically active material. The amount of top coatings on the stent may vary, but will generally be less than about 2000 μg, preferably the amount of top coating will be in the range of about 10 μg to about 1700 μg and most preferably in the range of from about 300 μg to about 1600 μg. Layering of coating of fast and slow hydrolyzing copolymers can be used to stage release of the drug or to control release of different agents placed in different layers. Polymer blends may also be used to control the release rate of different agents or to provide desirable balance of coating (i.e. elasticity, toughness etc.) and drug delivery characteristics (release profile). Polymers with different solubilities in solvents can be used to build up different polymer layers that may be used to deliver different drugs or control the release profile of a drug. For example since ε-caprolactone-co-lactide elastomers are soluble in ethyl acetate and ε-caprolactone-co-glycolide elastomers are not soluble in ethyl acetate. A

first layer of ε-caprolactone-co-glycolide elastomer containing a drug can be over coated with ε-caprolactone-co-glycolide elastomer using a coating solution made with ethyl acetate as the solvent. Additionally, different monomer ratios within a copolymer, polymer structure or molecular weights may result in different solubilities. For example, 45/55 ε-caprolactone-co-glycolide at room temperature is soluble in acetone whereas a similar molecular weight copolymer of 35/65 ε-caprolactone-co-glycolide is substantially insoluble within a 4 weight percent solution. The second coating (or multiple additional coatings) can be used as a top coating to delay the drug deliver of the drug contained in the first layer. Alternatively, the second layer could contain a different drug to provide for sequential drug delivery. Multiple layers of different drugs could be provided by alternating layers of first one polymer then the other. As will be readily appreciated by those skilled in the art numerous layering approaches can be used to provide the desired drug delivery.

[0030] The coatings can be used to deliver therapeutic and pharmaceutic agents such as, but not limited to: antiproliferative/antimitotic agents including natural products such as vinca alkaloids (i.e. vinblastine, vincristine, and vinorelbine), paclitaxel, epidipodophyllotoxins (i.e. etoposide, teniposide), antibiotics (dactinomycin (actinomycin D) daunorubicin, doxorubicin and idarubicin), anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin) and mitomycin, enzymes (L-asparaginase which systemically metabolizes L-asparagine and deprives cells which don't have the capacity to synthesize their own asparagine); antiproliferative/antimitotic alkylating agents such as nitrogen mustards (mechlorethamine, cyclophosphamide and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (hexamethylmelamine and thiotepa), alkyl sulfonates-busulfan, nirtosoureas (carmustine (BCNU) and analogs, streptozocin),trazenes - dacarbazinine (DTIC) ; antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate), pyrimidine analogs (fluorouracil, floxuridine, and cytarabine), purine analogs and related inhibitors (mercaptopurine, thioguanine, pentostatin and 2-chlorodeoxyadenosine{cladribine}) ; platinum coordination complexes (cisplatin, carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide; hormones (i.e.estrogen); Anticoaglants (heparin, synthetic heparin salts and other inhibitors of thrombin); fibrinolytic agents (such as tissue plasminogen activator, streptokinase and urokinase); antiplatelet:(aspirin, dipyridamole, ticlopidine, clopidogrel, abciximab); antimigratory; antisecretory (breveldin); antiinflammatory: such as adrenocortical steroids (cortisol, cortisone, fludrocortisone, prednisone, prednisolone, 6a-methylprednisolone, triamcinolone, betamethasone, and dexamethasone), non-steroidal agents (salicylic acid derivatives i.e. aspirin; para-aminophenol derivatives i.e. acetominophen; Indole and indene acetic acids (indomethacin, sulindac, and etodalac), heteroaryl acetic acids (tolmetin, diclofenac, and ketorolac), arylpropionic acids (ibuprofen and derivatives), anthranilic acids (mefenamic acid, and meclofenamic acid), enolic acids (piroxicam, tenoxicam, phenylbutazone, and oxyphenthatrazone), nabumetone, gold compounds (auranofin, aurothioglucose, gold sodium thiomalate); immunosuppressive: (cyclosporine, tacrolimus (FK-506), sirolimus (rapamycin), azathioprine, mycophenolate mofetil); Angiogenic: vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF); nitric oxide donors; anti-sense olgio nucleotides and combinations thereof.

[0031] Coating may be formulated by mixing one or more therapeutic agents with the coating polymers in a coating mixture. The therapeutic agent may be present as a liquid, a finely divided solid, or any other appropriate physical form. Optionally, the mixture may include one or more additives, e.g., nontoxic auxiliary substances such as diluents, carriers, excipients, stabilizers or the like. Other suitable additives may be formulated with the polymer and pharmaceutically active agent or compound. For example hydrophilic polymers selected from the previously described lists of biocompatible film forming polymers may be added to a biocompatible hydrophobic coating to modify the release profile (or a hydrophobic polymer may be added to a hydrophilic coating to modify the release profile). One example would be adding a hydrophilic polymer selected from the group consisting of polyethylene oxide, polyvinyl pyrrolidone, polyethylene glycol, carboxylmethyl cellulose, hydroxymethyl cellulose and combination thereof to an aliphatic polyester coating to modify the release profile. Appropriate relative amounts can be determined by monitoring the in vitro and/or in vivo release profiles for the therapeutic agents.

[0032] The best conditions for the coating application are when the polymer and pharmaceutic agent have a common solvent. This provides a wet coating that is a true solution. Less desirable, yet still usable are coatings that contain the pharmaceutic as a solid dispersion in a solution of the polymer in solvent. Under the dispersion conditions, care must be taken to ensure that the particle size of the dispersed pharmaceutical powder, both the primary powder size and its aggregates and agglomerates, is small enough not to cause an irregular coating surface or to clog the slots of the stent that we need to keep coating-free. In cases where a dispersion is applied to the stent and we want to improve the smoothness of the coating surface or ensure that all particles of the drug are fully encapsulated in the polymer, or in cases where we may want to slow the release rate of the drug, deposited either from dispersion or solution, we can apply a clear (polymer only) top coat of the same polymer used to provide sustained release of the drug or another polymer that further restricts the diffusion of the drug out of the coating. The top coat can be applied by dip coating with mandrel as previously described or by spray coating (loss of coating during spray application is less problematic for the clear topcoat since the costly drug is not included). Dip coating of the top coat can be problematic if the drug is more soluble in the coating solvent than the polymer and the clear coating redissolves previously deposited drug. The time spent in the dip bath may need to be limited so that the drug is not extracted out into the drug-free bath. Drying should be rapid so that the previously deposited drug does not completely diffuse into the topcoat.

[0033] The amount of therapeutic agent will be dependent upon the particular drug employed and medical condition being treated. Typically, the amount of drug represents about 0.001% to about 70%, more typically about 0.001% to about 60%, most typically about 0.001% to about 45% by weight of the coating.

[0034] The quantity and type of polymers employed in the coating layer containing the pharmaceutic agent will vary depending on the release profile desired and the amount of drug employed. The product may contain blends of the same or different polymers having different molecular weights to provide the desired release profile or consistency to a given formulation.

[0035] Absorbable polymers upon contact with body fluids including blood or the like, undergoes gradual degradation (mainly through hydrolysis) with concomitant release of the dispersed drug for a sustained or extended period (as compared to the release from an isotonic saline solution). Nonabsorbable and absorbable polymers may release dispersed drug by diffusion. This can result in prolonged delivery (over, say 1 to 2,000 hours, preferably 2 to 800 hours) of effective amounts (say, 0.001 $\mu g/cm^2$-min to 100 $\mu g/cm^2$-min) of the drug. The dosage can be tailored to the subject being treated, the severity of the affliction, the judgment of the prescribing physician, and the like.

[0036] Individual formulations of drugs and polymers may be tested in appropriate *in vitro* and *in vivo* models to achieve the desired drug release profiles. For example, a drug could be formulated with a polymer (or blend) coated on a stent and placed in an agitated or circulating fluid system (such as PBS 4% bovine albumin). Samples of the circulating fluid could be taken to determine the release profile (such as by HPLC). The release of a pharmaceutical compound from a stent coating into the interior wall of a lumen could be modeled in appropriate porcine system. The drug release profile could then be monitored by appropriate means such as, by taking samples at specific times and assaying the samples for drug concentration (using HPLC to detect drug concentration). Thrombus formation can be modeled in animal models using the ^{111} In-platelet imaging methods described by Hanson and Harker, Proc. Natl. Acad. Sci. USA 85:3184-3188 (1988). Following this or similar procedures, those skilled in the art will be able to formulate a variety of stent coating formulations.

Example 1

[0037] An absorbable elastomer based on 45:55 mole percent copolymer of ε-caprolactone and glycolide, with an IV of 1.58 (0.1 g/dl in hexafluoroisopropanol[HFIP] at 25°C) was dissolved five percent (5%) by weight in acetone and separately fifteen percent (15%) by weight in 1,1,2-trichloroethane. The synthesis of the elastomer is described in U. S. Patent 5,468,253 incorporated herein by reference. Gentle heating can be used to increase the dissolution rate. The high concentration coating could be formulated with or without pharmaceutical agent present. An initial primer coat of only the polymer is put on Cordis P-S 153 stent (commercially available from Cordis, a Johnson & Johnson Company) by dip coating in the five percent (5%) solution while the stent is placed on a 0.032 inch (0.81mm) diameter mandrel. The mandrel, with the stent on it, is removed from the dip bath and before the coating has a chance to dry the stent is moved along the length on the mandrel in one direction. This wiping motion applies high shear to the coating trapped between the stent and the mandrel. The high shear rate forces the coating out through the slots cut into the tube from which the stent is formed. This wiping action serves to force the coating out of the slots and keeps them clear. The "primed stent" is allowed to air dry at room temperature. The prime coat is about 100 micrograms of coating. After 1-2 hours of air drying, the stent is remounted on a 0.0355 inch (0.9mm) clean mandrel and dipped into a second, concentrated coat solution. This can be drug free or can contain about six percent (6%) by weight drug in addition to about fifteen percent (15%) polymer by weight in the coating solution. The dip and wipe process is repeated. The final coated stent is air dried for 12 hours and then put in a 60°C vacuum oven (at 30 in Hg vacuum) for 24 hours to dry. This method provides a coated stent with about 270 micrograms of polymer and about 180 micrograms of drug.

Example 2

[0038] This example describes experiments that demonstrate the ability of the dip and wipe coating approach to incorporate a bioactive agent in the coating and that the bioactive agent retains its biological activity. An initial primer coat of only the polymer described in Example 1 was placed on Cordis P-S 153 stent by dip coating in the five percent (5%) solution by weight while the stent is placed on a 0.032 inch (0.81mm) diameter mandrel. And primed as described in Example 1. The coated stent was then coated a second time with a coating solution of polymer and drug. The coated stent was dipped and wipe coated using the mandrel and a high concentration drug-polymer (15% polymer, 1:100 drug: polymer, and 2000 U/ml heparin-benzalkonium chloride [HBAC]; all in 70/30 acetone/DMSO) solution by the method described in Example 1. The HBAC coated stents had a total coating weight of about 350 micrograms. Coated stents were sent to North American Science Associtës Inc. (Northwood, Ohio USA) for a standard rabbit whole blood clotting time assay. The assay was performed by placing the stents on the surface of the Tryptic Soy Agar (TSA) plate along with a negative control sample (glass tubing) and a positive control (HBAC coated glass tubing). The 15 X 150 mm TSA plate was flooded with 35 ml of whole rabbit blood, obtained by arterial draw of a euthanized rabbit. The test

plate was incubated in ambient room temp. For 20-40 minutes. Following the incubation period, the samples were removed from the thrombus formed in the plate using forceps. The test and control sections were observed for evidence of adherence to the thrombus formation upon removal.

[0039] The heparinized stents were proven to be nonthrombogenic as compared with the non-heparinized controls.

Example 3

[0040] This example describes experiments that demonstrate the ability of the dip and wipe coating approach to provide coated stent with high coating loading and no bridging of the slots in the stent. A Cordis P-S 153 stent was taken and dip coated into a five percent (5%) solution of the elastomeric 45:55 mole percent of ε-caprolactone and glycolide copolymer (IV= 1.58) described in Example 1. The stent was removed and allow to air dry for 1-2 hours at room temperature. The coating added to the stent was about 100-150 micrograms. The slots in the stent were bridged with dry coating film (Figure 5). A second Cordis P-S 153 was dipped and wipe coated with the coating solution containing fifteen percent (15%) polymer as described in Example 1. The stent was found to have slots free of coating and to be loaded with 300 micrograms of coating. Similar experiments were performed with the Cordis Crown™ stent, the Guidant RX Multilink™ stent and the AVE GFX ™stent. The results were identical, dipping and wiping over a mandril allows high concentration coatings to provide high coating build on a variety of stents without the adverse effect of bridging the slots.

Example 4

[0041] This example demonstrates the differential solubility of elastomeric ε-caprolactone and glycolide copolymers and elastomeric ε-caprolactone and lactide copolymers in ethyl acetate. 0.2 g of ε-caprolactone and glycolide copolymer (45/55, IV=1.5, Tm ~62°C) were placed in a flat bottom glass vial along with 4 grams of ethyl acetate. These were heated to about 50°C on a hot plate with stirring bar over night. The result was partial solution with clear polymer on the walls and a cloudy solution at 50°C but the polymer precipitated out and coated the walls of the vial when the temperature came back to room temperature (~25°C), Similarly, 0.2 g of ε-caprolactone and lactide copolymer (40/60, IV=1.5, Tm ~ 132°C) were placed in a flat bottom glass vial with 4 g of ethyl acetate made in a manner similar to that described in Example 11. These were heated to about 50°C on a hot plate with stirring bar over night. The particles first swelled and then went into solution. On cooling to room temperature the solution remained clear and uniform.

Example 5

[0042] Multiple Dipping.

P-S stents were coated from a 5% w/w 45:55 ε-caprolactone and glycolide solution as described in the example 1. The initial coating resulted in ~ 100 micrograms of total solid on the stent. The stents were dried and then coated from a 15% w/w 45:55 ε-caprolactone and glycolide and 6% w/w drug solution. The second step resulted in ~ 170 micrograms of total solid and ~ 60 micrograms of drug on the stent. Stents were coated again from the same second solution and an increment of 30 micrograms (a total of 200 micrograms) of total solid and an increment of 20 micrograms of drug (a total of 80 micrograms) was observed. However when the dried stents were coated again with the same second solution total weight gain of the solid and the drug remain same.

Example 6

[0043] This Example describes applying a top coating to a coated stent with an ultrasonic spraying device.

[0044] A five percent by weight coating solution is made using 45:55 ε-caprolactone and glycolide described in Example 1 in a solvent solution of TCE :Acetone (1:1, w/w)

[0045] The ultrasonic spray unit is composed of a SonoTek (New York, U.S.A.) broadband ultrasonic generator (model 60-05108) attached to a nozzle (model 06-04010) an oscillated at 60KHz to generate a mean droplet size of 31 microns. The power at which the system was operated was is 5.8 mWatts. The flow rate was adjusted to about 0.3 ml/min. The ultrasonic spray system was placed in a plastic bag containment system to eliminate air currents and to slow evaporation. Stents would be positioned 1.5-5 cm distance from the nozzle and had a dwell time in the spray cloud of about 15-40 seconds.

[0046] The stent would then be dried in ambient conditions for 18-24 hours and subsequently vacuum dried at 60°C for 24 hours. Approximately, 100-150 micrograms of polymer was deposited per top coating run. A mandrel can be used to prevent coating the inside of the stent if desired.

Example 7

[0047] This Example describes the preparation of coated stents containing various levels of rapamycin for *in vitro* drug release testing.

[0048] 0.06 gms of Rapamycin was dissolved into 0.8 gms of 15% CAP/GLY solution in 1,1,2 TCE. The resulting coating solution contained 33.3% w/w drug on a dry, solid-only basis. Stents were coated by the method described in Example 1 and the coated stents were designated as `Std 33%'.

[0049] 0.015 gms of Rapamycin was dissolved into 0.5 gms of 18% CAP/GLY solution in 1,1,2 TCE. The resulting coating solution contained 14.3% w/w drug on a dry, solid-only basis. Stents were coated by the method described in Example 1 and the coated stents were designated as '14%'

[0050] 0.028 gms of rapamycin was dissolved into 0.5 gm of 18% CAP/GLY solution in 1,1,2 TCE. The resulting coating solution contained 23.7% w/w drug on a dry, solid-only basis. Stents were coated by the method described in Example 1. The dip-coated stents were spray coated with polymer-only solution as described in Example 6. The final coated stents were designated as '24-TC%'

[0051] 0.028 gms of rapamycin was dissolved into 0.5 gm of 18% CAP/GLY solution in 1,1,2 TCE. The resulting coating solution contained 23.7% w/w drug on a dry, solid-only basis. Stents were coated by the method described in Example 1. The dip-coated stents were spray coated with polymer-only solution as described in Example 6; However, a total volume of 200 microliters of spray solution was used in this case. The final coated stents were designated as '24- Thick TC%'

[0052] 0.06 gms of rapamycin was dissolved into 0.8 gm of 15% CAP/GLY solution in 1,1,2 TCE. The resulting coating solution contained 33.3% w/w drug on a dry, solid-only basis. Stents were coated by the method described in Example 1. The dip-coated stents were spray coated twice with ε-caprolactone-co-lactide (Cap/Lac) solution as described in Example 4. The final coated stents were designated as '33-TC%'.

[0053] 0.06 gms of rapamycin was dissolved into 0.8 gm of 15% CAP/LAC solution in 1,1,2 TCE. The resulting coating solution contained 33.3% w/w drug on a dry, solid-only basis. Stents were coated by the method described in example 1. The dip-coated stents were spray coated twice with polymer-only solution as described in Example 6 (except ε-caprolactone-co-lactide was used as the copolymer). The final coated stents were designated as '33-C/L TC%'.

Example 8

[0054] This example describes the results of testing the *in vitro* drug release of rapamycin from coated stent. Coated stents were prepared as described in Example 7 with varying concentrations of rapamycin were tested for the in vitro release of rapamycin into an aqueous ethanol solution. As is indicated in Figure 7, the stents denoted by the diamonds had a primer coating and a base coating that contained rapamycin. The total weight of the coating and rapamycin on the each stent was approximately 450 μg and contained 33 percent by weight of rapamycin. The coating was a copolymer of ε-caprolactone-co-glycolide (45:55 mole percent) applied by dip coating. The squares represent data points for stents having a primer coating and a base coating containing rapamycin. The total weight of the coating and drug was approximately 450 μg, which contained 14 percent by weight rapamycin. The coating material was also a copolymer of ε-caprolactone-co-glycolide (45:55 mole percent) applied by dip coating. The triangles represent data points for stents that had a primer coating and a base coating containing rapamycin. A primer coating and base coating (ε-caprolactone-co-glycolide 45:55 mole percent) were applied by dip coating the stent. A top coat of 200 μg (ε-caprolactone-co-glycolide 45:55 mole percent) was then applied using an ultrasonic spray device. The total weight of the coating and rapamycin was 650-700 μg, which contained 24 percent by weight rapamycin. The Xs represent data points for stents that had a primer coat and a base coating containing rapamycin. The primer coating and base coating (ε-caprolactone-co-glycolide 45:55 mole percent) were applied by dip coating the stent. A top coat of 100 μg (ε-caprolactone-co-glycolide 45:55 mole percent) was then applied using an ultrasonic spray device. The total weight of the coating and rapamycin was 550-600 μg, which contained 24 percent by weight rapamycin. The asterisk represents data points for stents that was coated with a primer, a base coat and two top coats. The primer coating and base coating (ε-caprolactone-co-glycolide 45:55 mole percent) were applied by dip coating the stents. A top coat of 100 μg (ε-caprolactone-co-glycolide; 45:55 mole percent) was then applied using an ultrasonic spray device. The total weight of the coating and rapamycin was approximately 550 μg, which contained 33 percent by weight rapamycin. The circles represent data points for stents that were dip coated with ε-caprolactone-co-lactide (40:60 mole percent). The stents were then top coated with an ultrasonic spray with approximately 100 μg of ε-caprolactone-co-lactide. The total coating weighed about 550 μg and contained 33 percent by weight rapamycin.

[0055] Each stent was placed in a 2.5mL of release medium (aqueous ethanol; 15 percent by volume at room temperature) contained in a 13 X 100 mm culture tube. The tube was shaken in a water bath (INNOVA™ 3100; New Brunswick Scientific) at 200 rpm while maintaining ambient conditions. After a given time interval (ranging froml5 minutes to one day) the tubes were removed from the shaker and the respective stents carefully transferred to a fresh 2.5

ml Aliquot of release medium. The new tube was placed on the shaker and agitation resumed. A sample was removed from the aliquot, which had previously contained the stent and placed in a HPLC vial for determination of the rapamycin content by HPLC.

[0056] The HPLC system used to analyze the samples was a Waters Alliance with a PDA 996. This system is equipped with a photodiode array detector. 20μL of each sample was withdrawn and analyzed on a C_{18}-reverse phase column (Waters Symmetry™ Column: 4.6mm X 100mm RP_{18} 3.5 μm with a matching guard column) using a mobile phase consisting of acetonitrile/methanol/water (38:34:28 v/v) delivered at a flow rate of 1.2 mL/min. The column was maintained at 60°C through the analysis. Under these analytical conditions rapamycin had a retention time of 4.75± 0.1 minutes. The concentration was determined from a standard curve of concentration versus response (area-under the curve) generated from rapamycin standards in the range of from 50ng/mL to 50μg/mL.

[0057] The results from testing the coated stents described above is shown in Figure 7.

<u>Example 9</u>

[0058] The goal of this study was to assess the rate of release of rapamycin from polymer-coated stents introduced in vivo into the coronary arteries of Yorkshire pigs. At various times after introduction of stents, the pigs were euthanized and the coronary arteries removed, the stents dissected free of the artery and analysed for rapamycin content using loading assay previously described. Through comparison with the amount of rapamycin contained on control, non-implanted stents, the *in vivo* rate of rapamycin release from the polymer coatings could be determined.

Experimental Procedure:

[0059] Male Yorkshire pigs weighing were used for these experiments. Animals were anesthetized with xylazine (2 mg/kg, IM), ketamine (17mg/kg, IM) and atropine (0.02 mg/kg IM). Pigs were then intubated using standard procedure, and placed on flow-by oxygen with 1-2.5% volatile isoflurane for maintenance anesthesia via the endotracheal tube. Peripheral intravenous access was achieved by insertion of a 20 gauge Angiocath into the marginal ear vein; a 20 gauge arterial catheter was also placed in the ear for continuous blood pressure and heart rate monitoring.

[0060] To minimize the chance for clot formation at the stent site, animals were started on oral aspirin 325 mg per day three days prior to the planned procedure. Upon confirmation of adequate depth of anesthesia, the right inguinal region was shaved and sterilized, and sterilely draped. Aseptic technique was used throughout the remainder of the procedure. A linear incision parallel to the femoral vessels was made and the subcutaneous tissues dissected to the level of the artery. After adequate exposure, the femoral artery was isolated proximally with umbilical tape and distally with a 3.0 silk tie for hemostasis. Using surgical scissors, an arteriotomy was made, and an 8 Fr sheath inserted in the artery. Heparin 4,000 units and bretylium 75 mg were then administered intravenously after sheath insertion. Electrocardiogram, respiratory pattern, and hemodynamics were continuously monitored.

[0061] A hockey stick guiding catheter was inserted via the femoral sheath, and advanced to the left coronary ostium, whereupon left coronary cineangiography was performed. A single frame anteroposterior radiogram was developed, and the luminal diameters of the left anterior descending and circumflex arteries measured, in order to size the balloon-stent assembly for a prespecified balloon-to-artery ratio of approximately 1.1 - 1.2:1. Using guide catheter support and fluoroscopic guidance, a 0.014" guidewire was advanced into the lumen of the left anterior descending artery. Intracoronary stenting was performed by advancing a stent mounted on a conventional angioplasty balloon into position in the mid-portion of the left anterior descending artery. The stent was deployed by inflating the mounting balloon to 8 atmospheres for 30 seconds. Upon confirmation of vessel patency, the balloon and guidewire were removed from the left anterior descending artery, and the identical procedure was performed in the left circumflex artery. Upon completion of stent delivery in the left circumflex artery, the balloon and guidewire were withdrawn.

[0062] The guiding catheter and femoral arterial sheath were then removed, the femoral artery tied proximally with 3-0 silk suture for hemostasis and the inguinal incision was closed. After discontinuation of anesthesia, were returned to colony housing. Daily aspirin 325 mg was continued until euthanasia.

[0063] At various times after stent implantation, euthanasia was performed by overdose of pentobarbital administered IV. The chest was opened via a mid-sternal incision and the heart removed. Both the LAD and LCX were carefully dissected free of surrounding tissue. The stent was then dissected free of the arterial tissue and placed in a vial. The arterial tissue was frozen and stored for later analysis by HPLC.

[0064] Figure 7 illustrates a typical *in vivo* release curve for a stent coating consisting of 33% rapamycin in polycaprolactone-co-glycolide.

<u>Example 10</u>

[0065] This Example describes the *in vivo* testing of coated stents in a porcine coronary artery model.

[0066] This preliminary study was conducted to assess the ability of rapamycin released from ε-caprolactone-co-glycolide copolymer-coated stents to inhibit intimal hyperplasia in vivo. Fourteen days after receiving rapamycin-loaded or control polymer coated stents, the male Yorkshire pigs were euthanized and the coronary arteries removed, the vessels prepared for histological evaluation and analysed for the amount of intimal growth. Through comparison control metal stents and stents containing polymer only, the in vivo ability of rapamycin to prevent neointimal growth could be determined.

[0067] Ethylene oxide-sterilized Palmaz-Schatz stents were implanted under sterile conditions in anesthetized farm pigs weighing 38 to 48 kg. Twenty-four hours prior to stent implantation, animals were given aspirin (325 mg, p.o., qd) and ticlopidine (250 mg, p.o., qd) to control chronic thrombosis; both aspirin and ticlopidine were continued daily until sacrifice. Anesthesia was induced with ketamine (20 mg/kg, i.m.), xylazine (2 mg/kg, i.m.) and sodium pentobarbital (10 mg/kg as needed) and maintained on 1-2% isofluorane in oxygen. An 8 Fr sheath was placed in an asceptically isolated left carotid artery and used subsequently to conduct either an 8 Fr JL 3.5 guide catheter for coronary angiography or to place a 0.014 inch guidewire for balloon delivery of stents to the appropriate coronary arteries. Heparin (150 unit/kg) was administered intraprocedurally to prevent acute thrombosis. Four experimental groups were employed; 1) metal stent control; 2) metal stent coated with 45/55 (w/w) ε-caprolactone glycolide copolymer (CAP/GLY); 3) 32 μg rapamycin/stent formulated in CAP/GLY; 4) 166 μg rapamycin/stent formulated in CAP/GLY. Stents were deployed in both the LAD and LCX coronary arteries. Angiography was performed prior to, during, and immediately after stenting to both size the vessel for choice of balloon diameter (3.0, 3.5 or 4.0 mm) and to obtain measurements for determination of the balloon/artery ratio. Stents were deployed by inflating the delivery balloon to 8-10 ATM for 30 sec. Angiography was also performed at 14 days post-implantation to obtain final vessel diameter. Treatment groups were randomized and individual stents were implanted by an investigator who was blinded as to the treatment. However, only one treatment was employed in any given pig. Fourteen days after implantation, animals were killed, the vessels were perfusion fixed for 10 minutes at 100 mmHg with 10% formalin and then stored in 10% buffered formalin.

[0068] For histological assessment, the stented vessel was embedded in glycol methacrylate. Four 3 - 5 μm thick cross-sections taken at equal intervals along the length of the stent were placed on glass slides and prepared with Miller's Elastin stain. Histomorphometric measurements were determined in each section *via* microscopy and computerized image analysis. Individual values obtained for each vessel represent the average of the 4 measured sections. Differences between treatments were assessed by ANOVA and Dunnett's test.

Table 1.

	Histology		*Angiography*	
Treatment	***Intima/Med ia ratio***	***Intimal Area (mm^2)***	***% Diameter Stenosis***	***B/A Ratio***
Metal Control (n=10)	0.90 ± 0.05	3.65 ± 0.82	24.8 ±3.9[1]	1.27 ± 0.05
CAP/GLY (n=8)	0.91 ± 0.11	4.15 ± 0.23	38.0 ± 4.0	1.32 ± 0.04
CAP/GLY ± 32 μg rapamycin (n=10)	0.75 ± 0.04	3.27 ± + 0.16	21.6 ± 3.6[1]	1.23 ± 0.03
CAP/GLY ± 166 μg rapamycin (n=8)	0.65 ± 0.04[1,2]	2.87 ± 0.31	23.9 ± 2.3[1]	1.27 ± 0.05

[1] $p<0.05$ from CAP/GLY
[2] $p<0.05$ from Metal Control

All values are mean±sem. B/A ratio = balloon to artery ratio, an index of the consistency of stent expansion from group to group

[0069] As can be seen in Table 1, local delivery of rapamycin to injured coronary arteries resulted in a significant ($p<0.05$) reduction in intima:media ratio in the 166 μg treatment group and a small but non-significant reduction in the 32 μg treatment group when compared with the polymer and bare metal control groups. Rapamycin delivered from the GAP/GLY coating also resulted in non-significant dose-related decreases in neointimal area in both the 32 μg and 166 μg treatment groups. The percent diameter stenosis as assessed by angiography was also significantly reduced in the 2 rapamycin treatment groups when compared to the CAP/GLY group, although the reduction in this parameter from the metal control was small and non-significant. Never-the-less, in this preliminary 14 day study, these data suggest that local release of rapamycin from a biodegradable hydrophobic polymer coating may be capable of limiting the amount of neointimal proliferation which occurs as a result of stent deployment.

Example 11

[0070] In the glove box, 100 µL (33 µmol) of a 0.33 M stannous octoate solution in toluene, 115 µL (1.2 mmol) of diethylene glycol, 24.6 grams (170 mmol) of L-lactide, and 45.7 grams (400 mmol) of ε-caprolactone were transferred into a silanized, flame dried, two neck, 250 mL round bottom flask equipped with a stainless steel mechanical stirrer and a nitrogen gas blanket. The reaction flask was placed in an oil bath already set at 190°C and held there. Meanwhile, in the glove box, 62.0 grams (430 mmol) L-lactide were transferred into a flame dried, pressure equalizing addition funnel. The funnel was wrapped with heat tape and attached to the second neck of the reaction flask. After 6 hours at 190°C, the molten L-lactide was added to the reaction flask over 5 minutes. The reaction was continued overnight for a total reaction time of 24 hours at 190°C. The reaction was allowed to cool to room temperature overnight. The copolymer was isolated from the reaction flask by freezing in liquid nitrogen and breaking the glass. Any remaining glass fragments were removed from the copolymer using a bench grinder. The copolymer was again frozen with liquid nitrogen and broken off the mechanical stirring paddle. The copolymer was ground into a tared glass jar using a Wiley Mill and allowed to warm to room temperature in a vacuum oven overnight. 103.13 grams of 40:60 poly(ε-caprolactone-co-L-lactide) were added to a tared aluminum pan and then devolitilized under vacuum at 110°C for 54 hours. 98.7 grams (95.7% by weight) of copolymer were recovered after devolitilization.

Claims

1. A method for coating a stent having an outer surface and inner surface with passages between the outer and inner surfaces comprising:

    (a) contacting the stent with a liquid coating solution containing a film forming biocompatible polymer under conditions suitable to allow the film forming biocompatible polymer to coat at least one surface of the stent;
    (b) before the coating solution dries creating fluid movement out of the passages of the stent sufficient to prevent the film forming biocompatible polymer from substantially blocking said passages thereafter;
    (c) drying the stent to provide at least a partially coated stent with a first coating.

2. The method of claim 1 wherein the stent is contacted with the coating solution by dipping the stent into the coating solution or by spraying the coating solution on to the stent.

3. The method of claim 1 or claim 2 wherein fluid movement is created: by contacting a mandrel with the inner surface of the stent and moving the mandrel relative to the stent to prevent bridges from forming in said passages; or by contacting the outer surface of the stent with the inner surface of a tube and moving the tube relative to the stent to prevent bridges from forming in said passages.

4. The method of any of claims 1 to 3 wherein the film forming biocompatible polymer is an aliphatic polyester, a poly (amino acid), a copoly(ether-ester), a polyalkylene oxalate, a polyamide, a poly(iminocarbonate), a polyorthoester, a polyoxaester, a polyamidoester, a polyoxaester containing amido groups, a poly(anhydride), a polyphosphazene, a biomolecule or a blend thereof.

5. The method of claim 4 wherein the film forming polymer is a biocompatible aliphatic polyester, which is preferably elastomeric.

6. The method of claim 5 wherein the biocompatible aliphatic polyester is an elastomeric copolymer of ε-caprolactone and glycolide, an elastomeric copolymer of ε-caprolactone and lactide, an elastomeric copolymer of p-dioxanone and lactide, an elastomeric copolymer of ε-caprolactone and p-dioxanone, an elastomeric copolymer of p-dioxanone and trimethylene carbonate, an elastomeric copolymer of trimethylene carbonate and glycolide, an elastomeric copolymer of trimethylene carbonate and lactide or blend thereof.

7. The method of any one of claims 1 to 6 wherein additionally contained in the coating solution is a pharmaceutically active compound.

8. The method of claim 7 wherein the pharmaceutically active compound is: an antiproliferative/antimitotic agent; an antibiotic; an enzyme; an antiproliferative/antimitotic alkylating agent; an antiproliferative/antimitotic antimetabolite; a hormone; an anticoaglant; a fibrinolytic agent; an antiplatelet agent; an antimigratory agent; an antisecretory agent; an antiinflammatory agent; an immunosuppressive agent; an angiogenic agent; a nitric oxide donor; an anti-

sense oligonucleotide or a combination thereof, and is preferably rapamycin.

9. The method of any one of claims 1 to 8, wherein additionally present is a biocompatble hydrophilic polymer.

10. The method of any one of claims 1 to 9 wherein after the stent is dried a second coating is applied.

FIG. 1

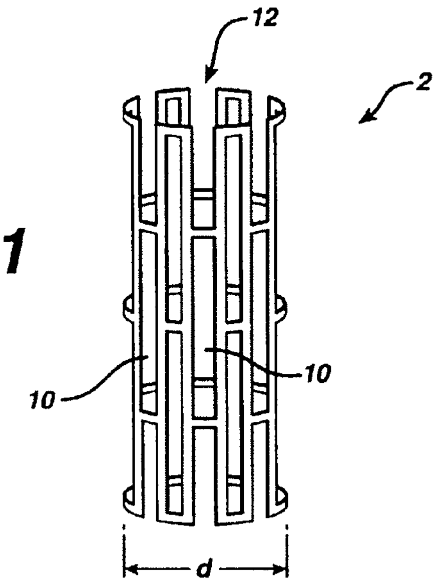


FIG. 2

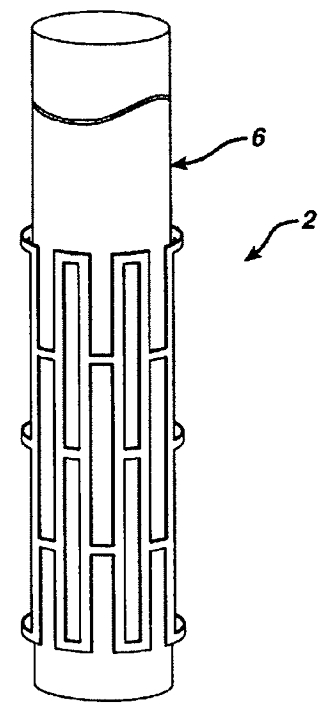


FIG. 3

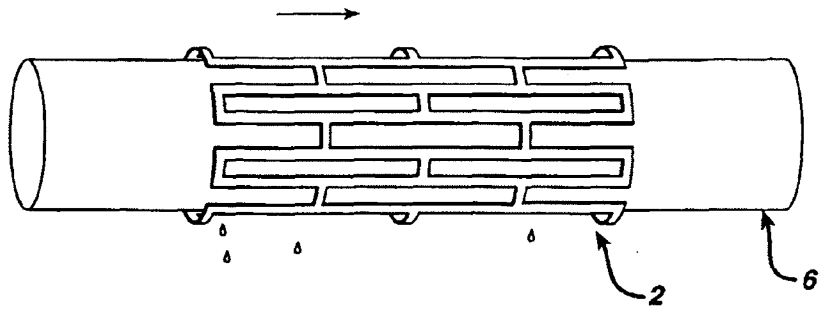


FIG. 4

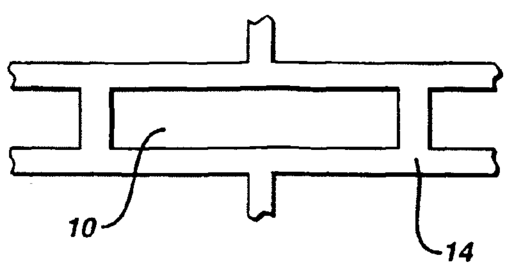


FIG. 5

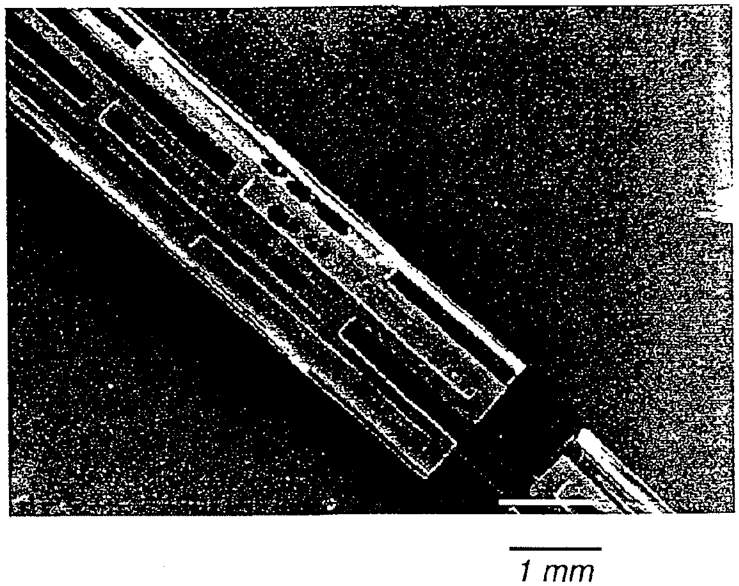


FIG. 6

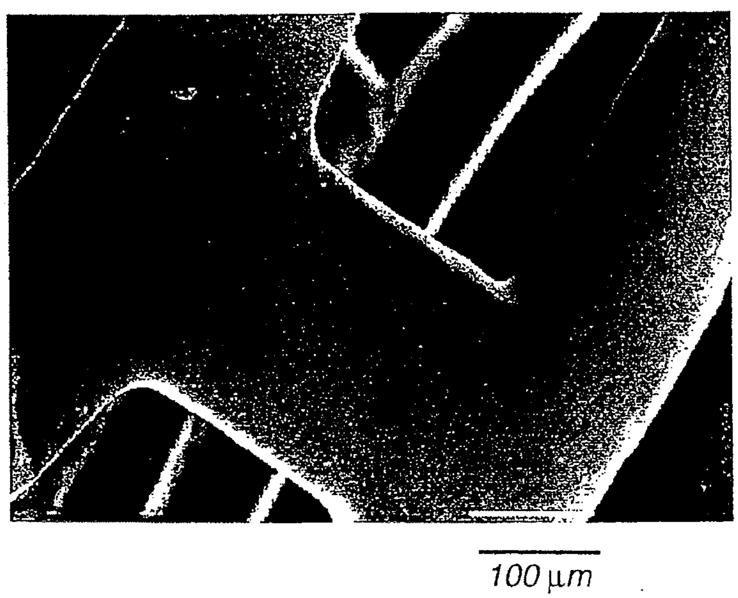
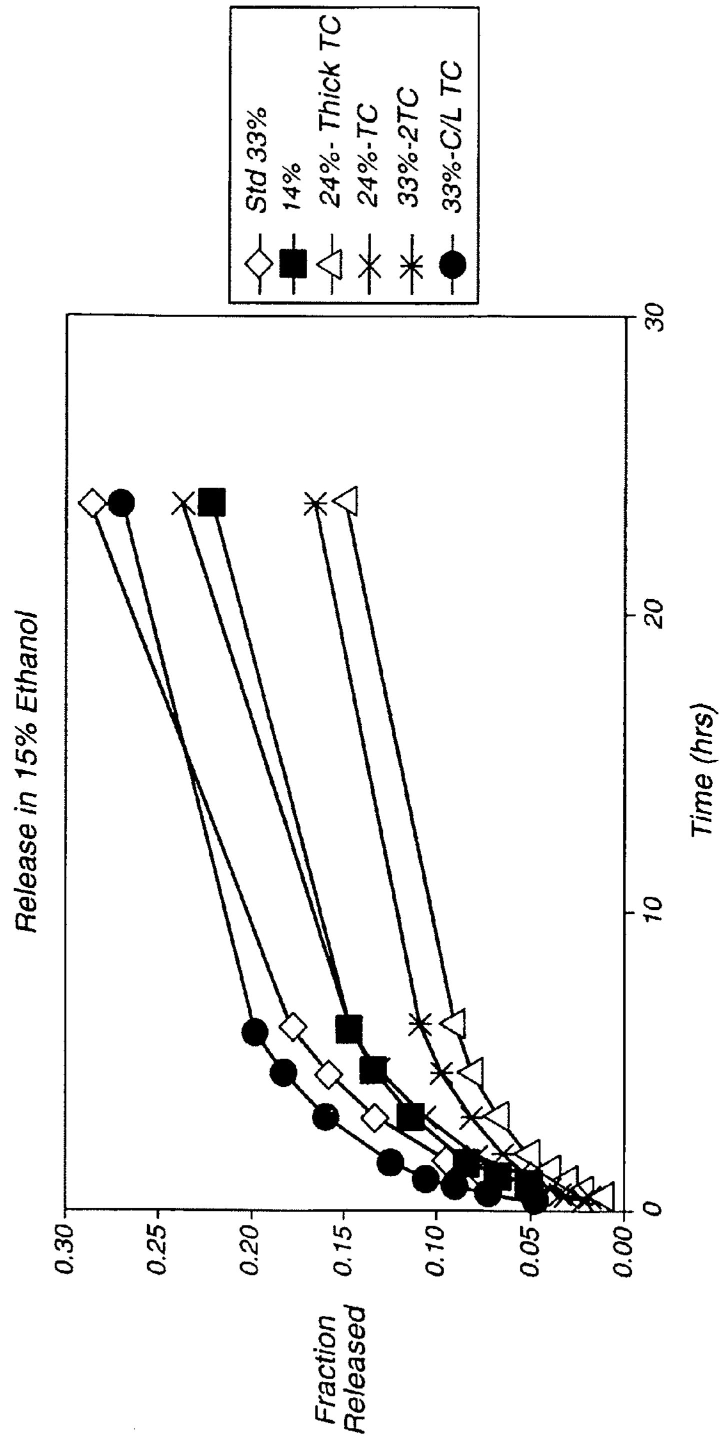


FIG. 7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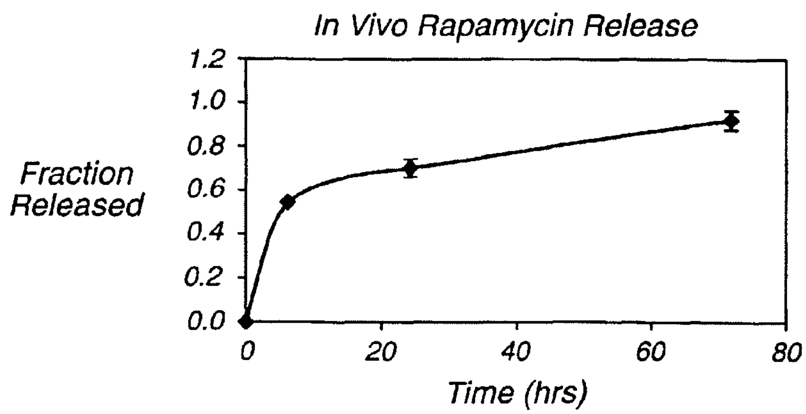
Fraction Released
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
Time (hrs)
Std 33%
14%
24%- Thick TC
24%-TC
33%-2TC
33%-C/L TC

FIG. 8


In Vivo Rapamycin Release
Fraction Released
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Time (hrs)

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(54) Process for coating stents

(57) A process is provided for coating stents having a first and second surface with passages there between to avoid blockage and bridging of the passages. The process comprises contacting the stent with a liquid coating solution containing a film forming biocompatible polymer under conditions suitable to allow the film forming biocompatible polymer to coat at least one surface of the stent while maintaining a fluid flow through said passages sufficient prevent the film forming biocompatible polymer from substantially blocking said passages. Also described are stents coated by this process.

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European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 99 30 5134

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 97 37617 A (JAYARAMAN SWAMINATHAN) 16 October 1997 (1997-10-16) * abstract * * page 10 - page 11 * * page 13 * * page 15 * * page 16 * * page 18; claims *	1-6,9,10	A61L31/16 A61L31/14 A61L31/10 A61L33/16 A61L33/14 A61L33/10
A	WO 97 10011 A (SCHNEIDER USA INC) 20 March 1997 (1997-03-20) * page 3, line 19-26 * * page 6, line 6-20 * * page 7, line 5-11 * * page 17, line 1-9 * * page 21, line 2-8 *	1,2,7,8	
A	US 5 534 287 A (LUKIC GORAN) 9 July 1996 (1996-07-09) * column 1, line 10-22 * * column 2 * * column 3, line 42 - column 4, line 39 *	1-3	TECHNICAL FIELDS SEARCHED (Int.Cl.7) A61L
A,D	US 5 700 583 A (JAMIOLKOWSKI DENNIS D ET AL) 23 December 1997 (1997-12-23) * abstract * * column 2, line 13-20 * * column 7 - column 9 *	1,2,4-9	
A	EP 0 832 655 A (SCHNEIDER USA INC) 1 April 1998 (1998-04-01) * abstract * * page 1, line 24-33 * * page 4, line 19-34,58 * * column 5, line 1,37-42 * * column 6, line 39-45 * * column 7, line 1-13 * * column 10, line 48-56 *	1,2,7-10	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	15 November 2000	Böhm, I

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

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15-11-2000

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9737617 A	16-10-1997	US 5725548 A	10-03-1998
		US 5713949 A	03-02-1998
		AU 2728097 A	29-10-1997
		CA 2250954 A	16-10-1997
		EP 1006938 A	14-06-2000
		US 5755781 A	26-05-1998
		US 5895407 A	20-04-1999
		US 5922393 A	13-07-1999
WO 9710011 A	20-03-1997	US 5837313 A	17-11-1998
		AU 703805 B	01-04-1999
		AU 6965296 A	01-04-1997
		BR 9610607 A	04-05-1999
		EP 1019107 A	19-07-2000
		JP 11500047 T	06-01-1999
		NO 981066 A	08-05-1998
		US 6120536 A	19-09-2000
US 5534287 A	09-07-1996	EP 0621015 A	26-10-1994
		AT 164056 T	15-04-1998
		CA 2114891 A,C	24-10-1994
		CA 2205533 A	24-10-1994
		CA 2206709 A	24-10-1994
		CA 2206712 A	24-10-1994
		CA 2255476 A	24-10-1994
		DE 69317548 D	23-04-1998
		DE 69317548 T	13-08-1998
		DK 621015 T	21-12-1998
		ES 2114964 T	16-06-1998
		JP 2914420 B	28-06-1999
		JP 7000529 A	06-01-1995
US 5700583 A	23-12-1997	US 5648088 A	15-07-1997
		US 5595751 A	21-01-1997
		US 5464929 A	07-11-1995
		EP 0841361 A	13-05-1998
		JP 10158377 A	16-06-1998
		US 5844017 A	01-12-1998
		AU 720550 B	01-06-2000
		AU 7053496 A	15-05-1997
		CA 2189520 A	07-05-1997
		EP 0771832 A	07-05-1997
		JP 9194579 A	29-07-1997
		US 5620698 A	15-04-1997
		EP 0771849 A	07-05-1997
		US 5607687 A	04-03-1997

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15-11-2000

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5700583 A		US 5618552 A	08-04-1997
		AU 712863 B	18-11-1999
		AU 4569796 A	19-09-1996
		BR 9600893 A	30-12-1997
		CA 2170941 A	07-09-1996
		EP 0731123 A	11-09-1996
		JP 8333443 A	17-12-1996
		US 6100346 A	08-08-2000
		US 5597579 A	28-01-1997
		US 5645850 A	08-07-1997
		US 5698213 A	16-12-1997
		US 5962023 A	05-10-1999
		US 5859150 A	12-01-1999
EP 0832655 A	01-04-1998	US 6120536 A	19-09-2000
		CA 2207659 A	13-12-1997
		JP 10052502 A	24-02-1998
		US 6099562 A	08-08-2000

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

US005256790A

United States Patent [19]

[11] Patent Number: 5,256,790

Nelson

[45] Date of Patent: Oct. 26, 1993

[54] **27-HYDROXYRAPAMYCIN AND DERIVATIVES THEREOF**

[75] Inventor: **Frances C. Nelson**, Yardley, Pa.

[73] Assignee: **American Home Products Corporation**, New York, N.Y.

[21] Appl. No.: **9,605**

[22] Filed: **Jan. 27, 1993**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 930,124, Aug. 13, 1992, abandoned.

[51] **Int. Cl.**[5] **C07D 498/16**; A61K 31/695

[52] **U.S. Cl.** **514/291**; 540/456

[58] **Field of Search** 540/456; 514/291

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,929,992 12/1975 Sehgal et al. .
3,993,749 11/1976 Sehgal et al. .
4,316,885 2/1982 Rakhit .
4,401,653 8/1983 Eng .
4,650,803 3/1987 Stella et al. .
4,885,171 12/1989 Sehgal et al. .
5,078,999 1/1992 Warner et al. .
5,080,899 1/1992 Sturm et al. .
5,091,389 2/1992 Ondeyka et al. .
5,100,883 3/1992 Schiehser .
5,100,899 3/1992 Calne .
5,102,876 4/1992 Caufield .
5,118,677 6/1992 Caufield .
5,118,678 6/1992 Kao et al. .
5,130,307 7/1992 Failli et al. .
5,138,051 8/1992 Hughes et al. .
5,147,877 11/1992 Goulet 540/456
5,151,413 9/1992 Caufield et al. .
5,169,851 12/1992 Hughes et al. .
5,177,203 1/1993 Failli et al. .
5,194,447 3/1993 Kao .

FOREIGN PATENT DOCUMENTS

507555A1 7/1992 European Pat. Off. .

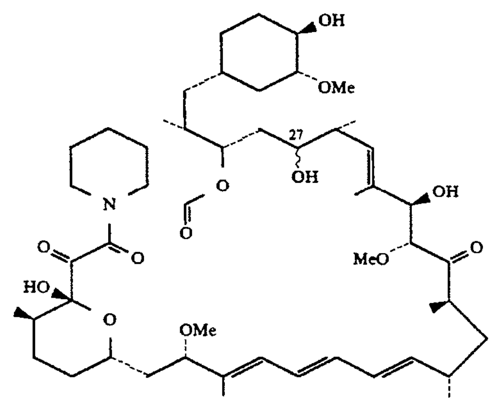
OTHER PUBLICATIONS

Venzina, C., J. Antibiot. 28:721 (1975).
Sehgal, S. N., J. Antibiot. 28:727 (1975).
Baker, H. J., Antibiot. 31:539 (1978).
Martel, R. R., Can. J. Physiol. Pharmacol. 55:48 (1977).
Staruch, M. J., FASEB 3:3411 (1989).
Dumont, F. J., FASEB 3:5256 (1989).
Calne, R. Y., Lancet 1183 (1978).
Morris, R. E., Med. Sci. Res. 17:877 (1989).
Meiser, B. J., J. Heart Lung Transplant. 11 (pt. 2):197 (1992).
Stepkowski, S. M., Transplantation Proc. 23:507–8 (1991).

Primary Examiner—Marianne M. Cintins
Assistant Examiner—John Peabody
Attorney, Agent, or Firm—Arnold S. Milowsky

[57] **ABSTRACT**

This invention provides a compound of formula I,



and 27-substituted derivatives thereof which are useful as immunosuppressive, antiinflammatory, antifungal, antitumor, and antiproliferative agents. The compound depicted by formula I is named 27-hydroxyrapamycin, and may also be referred to as 27-deoxo-27-hydroxyrapamycin.

11 Claims, No Drawings

27-HYDROXYRAPAMYCIN AND DERIVATIVES THEREOF

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation in part of Ser. No. 07/930,124, filed Aug. 13, 1992, now abandoned.

BACKGROUND OF THE INVENTION

This invention relates to a compound of formula I, which is named 27-hydroxyrapamycin, and derivatives thereof and a method for using them for inducing immunosuppression, and in the treatment of transplantation rejection, host vs. graft disease, autoimmune diseases, diseases of inflammation, solid tumors, fungal infections, and hyperproliferative vascular disorders.

Rapamycin is a macrocyclic triene antibiotic produced by *Streptomyces hygroscopicus*, which was found to have antifungal activity, particularly against *Candida albicans*, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S. N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31, 539 (1978); U.S. Pat. No. 3,929,992; and U.S. Pat. No. 3,993,749].

Rapamycin alone (U.S. Pat. No. 4,885,171) or in combination with picibanil (U.S. Pat. No. 4,401,653) has been shown to have antitumor activity. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); R. Y. Calne et al., Lancet 1183 (1978); and U.S. Pat. No. 5,100,899].

Rapamycin has also been shown to be useful in preventing or treating systemic lupus erythematosus [U.S. Pat. No. 5,078,999], pulmonary inflammation [U.S. Pat. No. 5,080,899], insulin dependent diabetes mellitus [Fifth Int. Conf. Inflamm. Res. Assoc. 121 (Abstract), (1990)], and smooth muscle cell proliferation and intimal thickening following vascular injury [Morris, R. J. Heart Lung Transplant 11 (pt. 2): 197 (1992)].

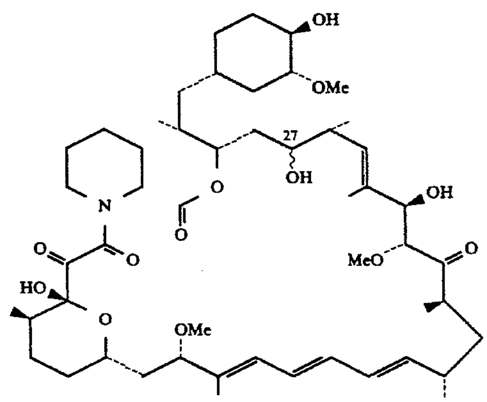
Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Pat No. 4,316,885) and used to make water soluble prodrugs of rapamycin (U.S. Pat. No. 4,650,803). Recently, the numbering convention for rapamycin has been changed; therefore according to Chemical Abstracts nomenclature, the esters described above would be at the 31- and 42- positions. Under the older numbering convention, 27-hydroxyrapamycin would be named as 24-hydroxyrapamycin.

U.S. Pat. No. 5,102,876 discloses 15-hydroxyrapamycin and 15,27-bis-hydroxyrapamycin, which were prepared by the reduction of rapamycin with diisobutylaluminum hydride, and a method of using them as immunosuppressive, antiinflammatory, and antifungal agents. 27-hydroxyrapamycin cannot be produced via the synthetic methodology disclosed in U.S. Pat. No. 5,102,876.

U.S. Pat. Nos. 5,138,051 and 5,169,851 disclose 33-hydroxyrapamycin which were prepared by the reduction of rapamycin using sodium triacetoxyborohydride, and a method of using them as immunosuppressive, antiinflammatory, and antifungal agents. 27-hydroxyrapamycin cannot be produced via the synthetic methodology disclosed in U.S. Pat. Nos. 5,138,051 and 5,169,851.

DESCRIPTION OF THE INVENTION.

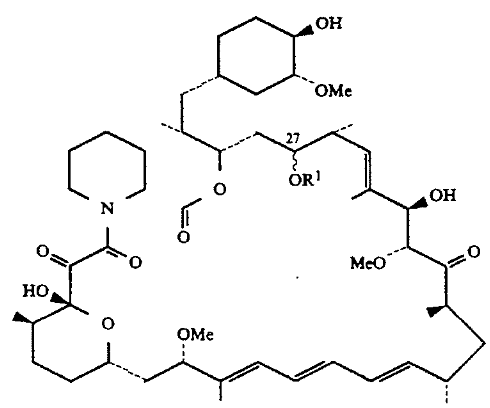
This invention provides a compound of formula I,



which is useful as an immunosuppressive, antiinflammatory, antifungal, antitumor, and antiproliferative agent. The compound depicted by formula I is named 27-hydroxyrapamycin, and may also be referred to as 27-deoxo-27-hydroxyrapamycin. 27-Hydroxyrapamycin may be administered orally, parenterally, intranasally, intrabronchially, transdermally, or rectally when administered in accordance with this disclosure.

This invention also provides derivatives of 27-hydroxyrapamycin which are useful as immunosuppressive, antiinflammatory, antifungal, antitumor, and antiproliferative agents having the formula II:

II



wherein
R^1 is

$$-\overset{\overset{O}{\|}}{C}R^2$$

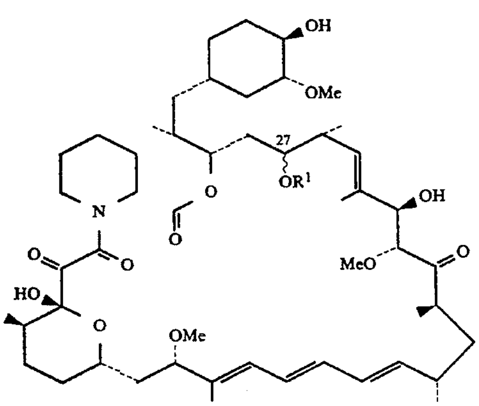
and

R^2 is alkyl of 1–10 carbon atoms, arylalkyl of 7–10 carbon atoms, or aryl wherein the aryl group may be optionally mono-, di-, or tri- substituted with a group selected from alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1–6 carbon atoms per alkyl group, alkylthio of 1–6 carbon atoms, $—SO_3H$, $—PO_3H$, and $—CO_2H$;

or a pharmaceutically acceptable salt thereof;

or having the formula III:

III



wherein

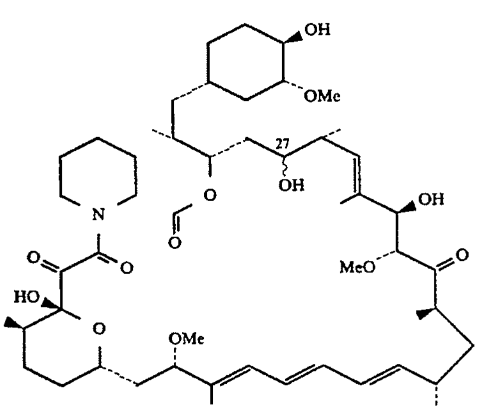
R^1 is

$$-\overset{\overset{O}{\|}}{C}R^2$$

and

R^2 is a mono-, di-, poly-, or per-fluorinated alkyl group of 1–10 carbon atoms; or having the formula IV:

IV



wherein

R^1 is

$$-\overset{\overset{O}{\|}}{C}R^2;$$

R^2 is

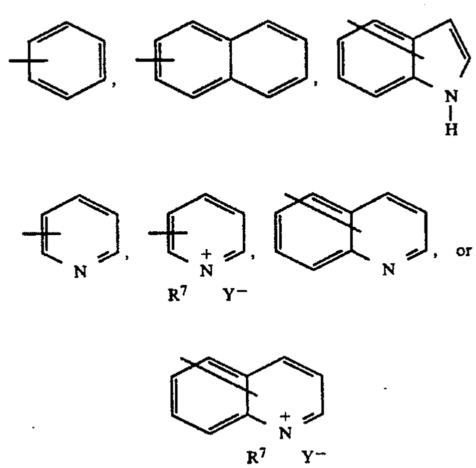
$$-X-\overset{\overset{O}{\|}}{C}-NR^3R^4;$$

X is $—(CH_2)_m—$ or $—Ar—$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–12 carbon atoms, $—(CH_2)_n—Ar$, $—(CH_2)_p—NR^5R^6$, or $—(CH_2)_p—N^+R^5R^6R^7Y^-$;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–12 carbon atoms, or $—(CH_2)_n—Ar$;

Ar is an optionally mono- or di- substituted group selected from



in which the optional substituents are selected from the group consisting of alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, or perfluoroalkyl of 1–6 carbon atoms;

R^7 is alkyl of 1–6 carbon atoms;

Y is a halide, sulfate, phosphate, or p-toluenesulfonate anion;

m = 1–6;

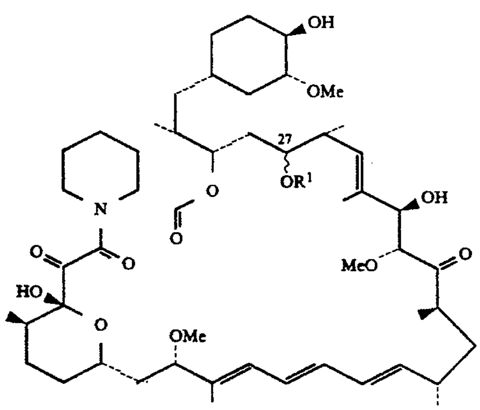
n = 1–6;

p = 1–6;

or a pharmaceutically acceptable salt thereof;

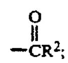
or having the formula V:

V



wherein

R^1 is



R^2 is $-NH(CR^3R^4)_n-X$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, cycloalkyl of 3-8 carbon atoms, halogen, or trifluoromethyl;

X is hydrogen, lower alkyl of 1-6 carbon atoms, cycloalkyl of 3-8 carbon atoms, trifluoromethyl, nitro, alkoxy of 1-6 carbon atoms, carboalkoxy of 2-7 carbon atoms, arylalkyl of 7-10 carbon atoms, halo, dialkylamino of 1-6 carbon atoms per alkyl group, thioalkyl of 1-6 carbon atoms, or Y;

Y is a phenyl group which may be optionally mono-, di-, or tri- substituted with a group selected from alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, alkoxy of 1-6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, dialkylamino of 1-6 carbon atoms per alkyl group, or alkylthio of 1-6 carbon atoms, $-SO_3H$, $-PO_3H$, and $-CO_2H$;

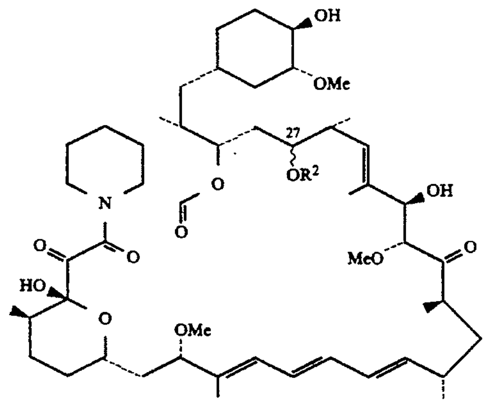
$n=0-5$;

with the proviso that R^1 and R^2 are not both hydrogen and when $n=0$, X is lower alkyl of 1-6 carbon atoms, cycloalkyl of 3-8 carbon atoms, arylalkyl of 7-10 carbon atoms, or Y;

or a pharmaceutically acceptable salt thereof;

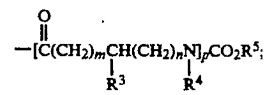
or having the formula VI:

VI



wherein

R^2 is



R^3 is hydrogen, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, $-(CH_2)_qCO_2R^6$, $-(CH_2)_rNR^7CO_2R^8$, carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazoylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or $-CO_2H$;

R^4 and R^7 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms;

R^5, R^6, and R^8 are each, independently, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or $-CO_2H$;

m is 0-4;

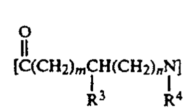
n is 0-4;

p is 1-2;

q is 0-4;

r is 0-4;

wherein R^3, R^4, m, and n are independent in each of the

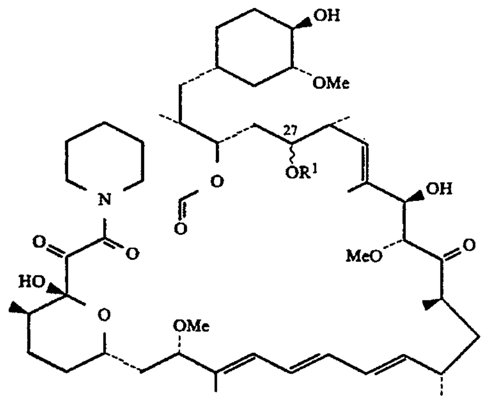


subunits when $p=2$;

or a pharmaceutically acceptable salt thereof;

or having the formula VII:

VII



wherein

R^1 is alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, $-CH_2YX$, $-C(CH_3)_2YX$, $-CH(CH_3)YX$, or L;

Y is O or S;

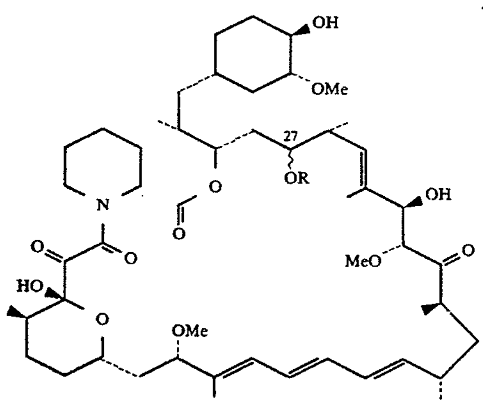
X is $-CH_3$, $-(CH_2)_nCH_3$, $-CH_2Ar$, $-(CH_2)_2OCH_3$, $-CH_2CCl_3$, $-CH(CH_3)_2$, or $-CH_2CH_2SiMe_3$;

L is tetrahydrofuran-2-yl, tetrahydrothiophen-2-yl, tetrahydrothiopyran-2-yl, tetrahydropyran-2-yl, 4-methoxytetrahydropyran-2-yl, 4-methoxytetrahydrothiopyran-2-yl, or 4-methoxytetrahydrothiopyran-2-yl S,S dioxide; and

$n=1-5$;

or having the formula VIII:

VIII



wherein

R is

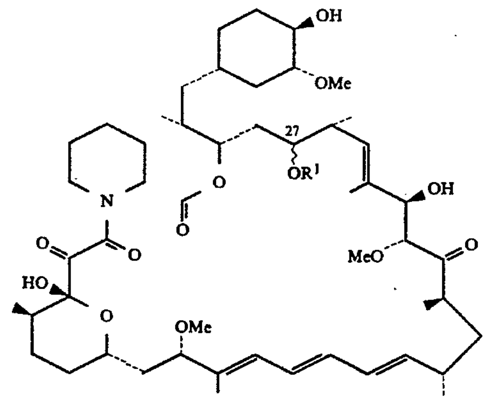
$$-\overset{O}{\overset{\|}{C}}(CH_2)_mNR^1R^2;$$

R^1 and R^2 are each hydrogen or alkyl of 1-3 carbon atoms or R^1 and R^2 together with the nitrogen to which they are attached form a saturated heterocyclic ring having 4-5 carbon atoms; and

$m=1-3$ or a pharmaceutically acceptable salt thereof;

or having the formula IX:

IX



wherein

R^1 is $-CONHSO_2-Ar$; and

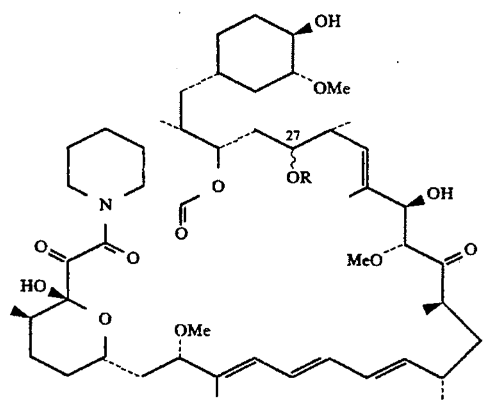
Ar is phenyl, naphthyl, pyridyl, quinolyl, isoquinolyl, quinoxalyl, thienyl, thionaphthyl, furyl, benzofuryl, benzodioxyl, benzoxazolyl, benzoisoxazolyl, or benzodioxolyl; wherein the Ar group may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, alkoxy of 1-6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1-6 carbon atoms per alkyl group, alkylthio of 1-6 carbon atoms, $-SO_3H$, $-PO_3H$, and $-CO_2H$;

or a pharmaceutically acceptable salt thereof when the Ar group contains a basic nitrogen or when the Ar group is substituted by dialklyamino of 1-6 carbon atoms per alkyl group, $-SO_3H$, $-PO_3H$, or $-CO_2H$;

or a pharmaceutically acceptable salt thereof;

or having formula X:

X



wherein

R is $-SO_2R^1$;

R^1 is alkyl, alkenyl, alkynyl containing 1 to 6 carbon atoms; or an aromatic moiety selected from the group consisting of phenyl and naphthyl or a heterocyclic moiety selected from the group consisting of thiophenyl and quinolinyl; or $-NHCOR^2$; and

R^2 is lower alkyl containing 1 to 6 carbon atoms;

or a pharmaceutically acceptable salt thereof.

Pharmaceutically acceptable salts may be formed from the compounds of formulas II, IV–VI, and VII–X from organic and inorganic acids and inorganic cations. Preferred organic and inorganic acids are those such as acetic, lactic, citric, tartaric, succinic, maleic, malonic, gluconic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, and the like. Preferred inorganic cations are those such as sodium, potassium, and the like. Based on this disclosure, other pharmaceutically acceptable salts that can be formed will be readily apparent to one skilled in the art.

When any of the compounds of formulas II–X contain an aryl or arylalkyl moiety, it is preferred that the aryl portion is a phenyl, naphthyl, pyridyl, quinolyl, isoquinolyl, quinoxalyl, thienyl, thionaphthyl, furyl, benzofuryl, benzodioxyl, benzoxazolyl, benzoisoxazolyl, or benzodioxolyl group that may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1–6 carbon atoms per alkyl group, alkylthio of 1–6 carbon atoms, $—SO_3H$, $—PO_3H$, and $—CO_2H$. It is more preferred that the aryl moiety is a phenyl group that is optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1–6 carbon atoms per alkyl group, alkylthio of 1–6 carbon atoms, $—SO_3H$, $—PO_3H$, and $—CO_2H$.

For the compounds of formula IV, preferred members are those in which X is $—(CH_2)_m—$ and R^3 and R^4 are alkyl of 1–6 carbon atoms; and those in which X is $—(CH_2)_m—$, R^3 is hydrogen, and R^4 is and Ar is $—(CH_2)_n—Ar$.

For the compounds of formula V, preferred members are those in which n is 0 and X is Y.

For the compounds of formula VI, preferred members are those in which m=0, n=0, and p=1; m=0, n=0, and p=2; n=0, and R^3 is $—(CH_2)_qCO_2R^6$; m=0, n=0, and R^3 is $—(CH_2)_rNR^7CO_2R^8$; and m=0, n=0, and R^3 is hydrogen.

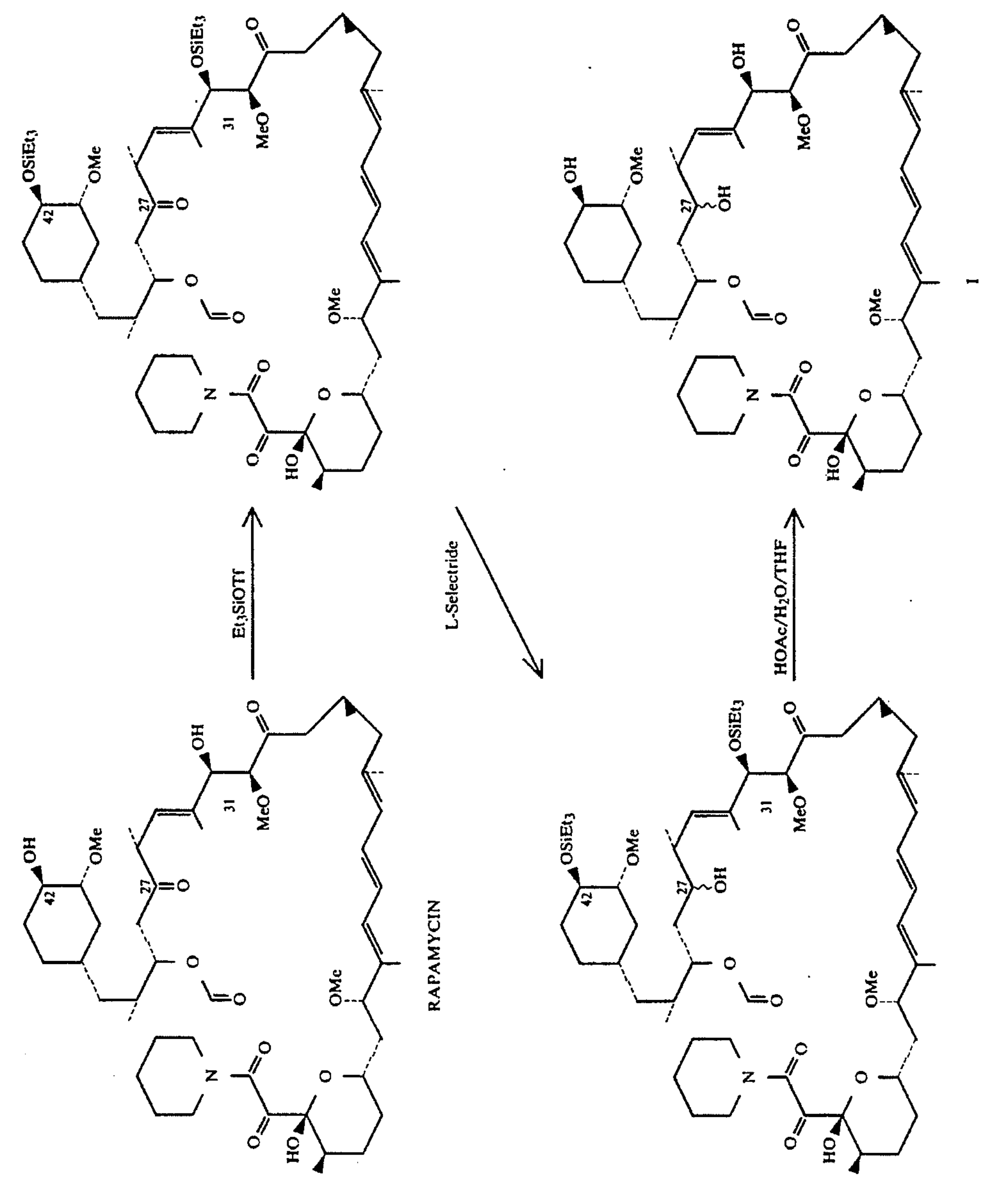
For the compounds of formula VII, preferred members include those in which Y is O and those in which R^1 is alkyl of 1–6 carbon atoms.

The compounds of formulas II–X may be administered orally, parenterally, intranasally, intrabronchially, transdermally, or rectally when administered in accordance with this disclosure.

This invention also provides pharmaceutical compositions comprising an effective amount of 27-hydroxyrapamycin or any of the compounds of formulas II–X, and a pharmaceutical carrier.

It is contemplated that when the compounds of this invention are used as an immunosuppressive or antiinflammatory agent, they can be administered in conjunction with one or more other immunoregulatory agents. Such other antirejection chemotherapeutic agents include, but are not limited to azathioprine, corticosteroids, such as prednisone and methylprednisolone, cyclophosphamide, rapamycin, cyclosporin A, FK-506, OKT-3, and ATG. By combining 27-hydroxyrapamycin or a derivative thereof with such other drugs or agents for inducing immunosuppression or treating inflammatory conditions, the lesser amounts of each of the agents are required to achieve the desired effect. The basis for such combination therapy was established by Stepkowski whose results showed that the use of a combination of rapamycin and cyclosporin A at subtherapeutic doses significantly prolonged heart allograft survival time. [Transplantation Proc. 23: 507 (1991)].

The preparation of 27-hydroxyrapamycin can be accomplished by the sequence of reactions shown below, beginning with rapamycin.


Et$_3$SiOTf
L-Selectride
HOAc/H$_2$O/THF
RAPAMYCIN
I

The 31- and 42-hydroxyl groups of rapamycin are first protected with a suitable protecting group, such as the triethylsilyl ether protecting group. Protection of the hydroxyl groups prior to reduction appears to be necessary to prevent overreduction and ring degradation. Reduction of the 27-ketone was selectively accomplished with L-Selectride (lithium tri-sec-butylborohydride) to provide a compound which was named 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin. Attempted reduction with DIBAL, as disclosed in U.S. Pat. No. 5,102,876, failed to provide any products in which the 27-ketone was the only keto-group that was reduced. Removal of the silyl ether protecting groups was accomplished under mildly acidic conditions, such as with a mixture of acetic acid, water, and THF. Removal of the silyl ether protecting groups can also be accomplished using fluoride ion generating reagents, such as hydrogen fluoride/pyridine. It is also contemplated that the 31- and 42-hydroxyl groups can be protected with other silylating reagants, such as triisopropylsilyl chloride or t-butyldimethylsilyl chloride, to allow selective reduction of the 27-ketone of rapamycin.

The derivatives of 27-hydroxyrapamycin that are claimed as part of this invention can be prepared by reacting the intermediate 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin with suitable electrophilic agents. The 27-acyl derivatives of formula II can be prepared by the method used in Examples 4 and 5. The 27-acyl derivative of formula II can also be prepared by reacting the 27-hydroxyl group of 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin with an acylating agent according the method described in U.S. Pat. No. 4,316,885, the disclosure of which is hereby incorporated by reference, followed by deprotection according to Examples 3 or 5.

The 27-fluorinated esters of formula III can be prepared by reacting the 27-hydroxyl group of 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin with a suitable fluorinated acylating agent as described in U.S. Pat. No. 5,100,883, the disclosure of which is hereby incorporated by reference, followed by deprotection according to Examples 3 or 5.

The 27-amide esters of formula IV can be prepared by acylating the 27-hydroxyl group of 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin with a suitable acylating agent as described in U.S. Pat. No. 5,118,677, the disclosure of which is hereby incorporated by reference, followed by deprotection according to Examples 3 or 5.

The 27-carbamates of formula V can be prepared by carbamylating the 27-hydroxyl group of 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin with a suitable carbamylating agent as described in U.S. Pat. No. 5,118,678, the disclosure of which is hereby incorporated by reference, followed by deprotection according to Examples 3 or 5.

The 27-aminoesters of formula VI can be prepared by acylating the 27-hydroxyl group of 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin with a suitable acylating agent as described in U.S. Pat. No. 5,130,307, the disclosure of which is hereby incorporated by reference, followed by deprotection according to Examples 3 or 5.

The 27-ethers of formula VII can be prepared by reacting the 27-hydroxyl group of 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin with a suitable acetal forming reagent as described in U.S. Pat. No. 5,151,413, the disclosure of which is hereby incorporated by reference, followed by deprotection using hydrogen fluoride/pyridine according to standard literature procedures. The alkyl or arylalkyl ethers of formula VII can be formed by alkylating the 27-hydroxyl group of 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin with a suitable alkylating agent, such as with an alkyl halide in pyridine.

The 27-aminoacyl compounds of formula VIII can be prepared by acylating the 27-hydroxyl group of 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin with a suitable acylating agent as described in U.S. Pat. No. 4,650,803, the disclosure of which is hereby incorporated by reference, followed by deprotection according to Examples 3 or 5.

The 27-sulfonylcarbamates of formula IX can be prepared by carbamylating the 27-hydroxyl group of 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin with a suitable carbamylating agent as described in U.S. patent application Ser. No. 07/837,048, filed Feb. 18, 1992, the disclosure of which is incorporated by reference, followed by deprotection according to Examples 3 or 5.

The 27-sulfonates and sulfamates of formula X can be prepared by reacting the 27-hydroxyl group of 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin with a suitable sulfonyl halide or (carboxysulfamoyl)triethylammonium hydroxide inner salt as described in U.S. Pat. No. 5,177,203, the disclosure of which is hereby incorporated by reference, followed by deprotection according to Examples 3 or 5.

Based on this disclosure, other derivatives of 27-hydroxyrapamycin will be apparent to one skilled in the art. For example, it is contemplated that other esters of the 27-hydroxyl group can be prepared. These include both organic esters and inorganic esters, such as phosphate, nitrate, sulfinate, sulfonate esters, and the like, and organic esters of these inorganic acids. These compounds are also expected to have a similar activity profile to the compounds of this invention. Additionally, the 27-hydroxyl group may be protected with suitable protecting groups, such as a silyl ether, to provide a 27,31,42-tris-silyl ether of 27-hydroxyrapamycin.

Immunosuppressive activity for representative compounds of this invention was evaluated in an in vitro standard pharmacological test procedure to measure lymphocyte proliferation (LAF) and in an in vivo standard pharmacological test procedure which evaluated the survival time of a pinch skin graft.

The comitogen-induced thymocyte proliferation procedure (LAF) was used as an in vitro measure of the immunosuppressive effects of representative compounds. Briefly, cells from the thymus of normal BALB/c mice are cultured for 72 hours with PHA and IL-1 and pulsed with tritiated thymidine during the last six hours Cells are cultured with and without various concentrations of rapamycin, cyclosporin A, or test compound. Cells are harvested and incorporated radioactivity is determined. Inhibition of lymphoproliferation is assessed as percent change in counts per minute from nondrug treated controls. The results are expressed as an IC_{50}.

A representative compound of this invention was also evaluated in an in vivo test procedure designed to determine the survival time of pinch skin graft from male DBA/2 donors transplanted to male BALB/c recipients. The method is adapted from Billingham R. E. and Medawar P. B., J. Exp. Biol. 28:385-402, (1951).

Briefly, a pinch skin graft from the donor is grafted on the dorsum of the recipient as a homograft, and an autograft is used as control in the same region. The recipients are treated with either varying concentrations of cyclosporin A as test control or the test compound, intraperitoneally. Untreated recipients serve as rejection control. The graft is monitored daily and observations are recorded until the graft becomes dry and forms a blackened scab. This is considered as the rejection day. The mean graft survival time (number of days ±S.D.) of the drug treatment group is compared with the control group.

The following table summarizes the results of representative compounds of this invention in these three standard test procedures.

TABLE 1

Compound	LAF (IC_{50})	Skin Graft (days ± SD)
27-Hydroxyrapamycin	3.7 nM	8.5 ± 1.2*
		8.17 ± 0.75*
		8.00 ± 0.63*
		9.17 ± 0.98+
		9.17 ± 0.75+
Example 5	99 nM#	
Rapamycin	4.8 nM	12.0 ± 1.7*
No Treatment		7.2 ± 0.45

*Evaluated in the skin graft procedure at a dose of 4 mg/kg.
+Evaluated in the skin graft procedure at a dose of 16 mg/kg.
#94% inhibition of T-cell proliferation at 1 μM and 69% inhibition at 0.1 μM.

The results of these standard pharmacological test procedures demonstrate immunosuppressive activity both in vitro and in vivo for the compounds of this invention. The results obtained in the LAF test procedure indicates suppression of T-cell proliferation. As a transplanted pinch skin grafts are typically rejected within 6–7 days without the use of an immunosuppressive agent, the increased survival time of the skin graft when treated with the compounds of this invention further demonstrates their utility as immunosuppressive agents.

Because the compounds of this invention are structurally similar to rapamycin and have a similar activity profile to rapamycin, the compounds of this invention also are considered to have antitumor, antifungal, and antiproliferative activities.

As such, the compounds of this invention are useful in the treatment of transplantation rejection such as, heart, kidney, liver, bone marrow, and skin transplants; autoimmune diseases such as lupus, rheumatoid arthritis, diabetes mellitus, myasthenia gravis, and multiple sclerosis; diseases of inflammation such as psoriasis, dermatitis, eczema, seborrhea, inflammatory bowel disease, and eye uveitis; solid tumors; fungal infections; and hyperproliferative vascular diseases such as restenosis.

Additionally, 27-Hydroxyrapamycin was found to have a half life of 17.5 hours in 0.1M phosphate buffer (pH 7.4, 37° C.) whereas rapamycin was found to have a half life of 11.8 hours under the same conditions. Therefore, by virtue of the reduced ketone at the 27-position, 27-hydroxyrapamycin provides an advantage over rapamycin by preventing degredative ring opening reactions, thus resulting in a more stable compound. The 27-hydroxyrapamycin derivatives of formulas II–X are also expected to resist ring degredative reactions better than rapamycin and 31- and/or 42-substituted rapamycin derivatives of rapamycin. The half life of 27-hydroxyrapamycin-27-ester with acetic acid in 0.1M phosphate buffer (pH 7.4, 37° C.) is 34 hours.

As 27-hydroxyrapamycin and the compound of Example V was prepared via its 31,42-silylated intermediate (Example 2), the compound of Example 2 is therefore useful as an intermediate of these two compounds. Additionally, 31,42-Bis-triethylsilyl ether of 27-hydroxyrapamycin-27-ester with acetic acid is also a useful intermediate in the preparation of the compound of Example 5.

The compounds of this invention can be formulated neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compounds of this invention can also be administered orally either in liquid or solid composition form.

The compounds of this invention may be administered rectally in the form of a conventional suppository.

For administration by intranasal or intrabronchial inhalation or insufflation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. The compounds of this invention may also be administered transdermally through the use of a transdermal patch containing the active compound and

a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermiable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

In addition, the compounds of this invention may be employed as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles containing 0.1-5 percent, preferably 2%, of active compound which may be administered to a fungally affected area.

The dosage requirements vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Based on the results obtained in the standard pharmacological test procedures, projected daily intravenous dosages of the compounds of this invention would be 0.001-25 mg/kg, preferably between 0.005-5 mg/kg, and more preferably between 0.01-0.5 mg/kg. Projected daily oral dosages of the compounds of this invention would be 0.005-75 mg/kg, preferably between 0.01-50 mg/kg, and more preferably between 0.05-10 mg/kg.

Treatment will generally be initiated with small dosages less than the optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached; precise dosages for oral, parenteral, intranasal, intrabronchial, transdermal, or rectal administration will be determined by the administering physician based on experience with the individual subject treated. In general, the compounds of this invention, are most desirably administered at a concentration that will generally afford effective results without causing any harmful or deleterious side effects.

The following examples illustrate the preparation of representative compounds of this invention.

EXAMPLE 1

31,42-Bis-triethylsilyl ether of rapamycin

To a solution of rapamycin (2 g, 2.18 mmol) in CH_2Cl_2 (10.9 mL) at 0° C. was added 2,6-lutidine (1.17 mL, 10.1 mmol) and triethylsilyl trifluoromethanesulfonate (1.13 mL, 5.03 mmol) dropwise. The reaction was stirred at 0° C. for an additional 45 min, allowed to warm to room temperature, and stirred overnight. The reaction was then quenched with $NaHCO_3$ and diluted with ethyl acetate. The organic layer was separated and washed with 2N HCl, $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed using hexane-ethyl acetate (4:1) as eluant to provide 1.04 g (42%) of 31,42-bis-triethylsilyl ether of rapamycin.

IR (KBr) 3500 (m, br), 2925 (s), 2875 (s), 1720 (s), 1640 (s), 1450 (s), 1370 (w), 1235 (w), 1185 (w), 1100 (s), 980 (m), 815 (m), 745 (m); ^1H NMR (400 MHz, $CDCl_3$) δ0.44-0.50 (comp m, 6H), 0.52-0.60 (comp m, 6H), 0.67 (m, 1H), 0.82-0.96 (comp m, 24H), 1.00-1.04 (comp m, 9H), 1.06-1.25 (comp m, 4H), 1.30-1.60 (comp m, 12H), 1.61, 1.64 (d, rotamers, J=3.74, 0.80 Hz, 3H), 1.68-1.83 (comp m, 5H), 1.72, 1.74 (d, rotamers, J=1.04 Hz, 3H), 1.96 (m, 1H), 2.25 (m, 2H), 2.32 (dd, J=3.00, 15.88 Hz, 1H), 2.58 (dd, J=8.09, 16.00 Hz, 1H), 2.68 (m, 1H), 2.87 (m, 1H), 3.10, 3.11 (s, rotamers, 3H), 3.24 (s, 3H), 3.33 (m, 3H), 3.37, 3.39 (s, rotamers, 3H), 3.68 (m, 2H), 3.75 (m, 1H), 3.82 (d, J=6.23 Hz, 1H), 4.10 (d, J=5.60 Hz, 1H), 4.68 (d, J=1.66 Hz, 1H), 5.00 (m, 1H), 5.20 (d, J=10.17 Hz, 1H), 5.28 (d, J=4.57 Hz, 1H), 5.53 (dd, J=8.20, 15.05 Hz, 1H), 6.02 (dd, J=1.04, 10.79 Hz, 1H), 6.14 (m, 1H), 6.34 (qd, J=10.48, 28.94 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ4.63, 4.72, 5.01, 6.68, 6.72, 6.79, 10.14, 12.33, 13.72, 14.94, 15.42, 16.06, 21.46, 25.14, 26.86, 27.31, 31.29, 31.82, 32.97, 33.88, 33.98, 34.08, 35.24, 36.15, 38.60, 38.67, 39.87, 41.70, 42.44, 44.03, 47.03, 51.25, 55.78, 58.07, 58.15, 66.92, 75.61, 79.26, 84.05, 84.11, 84.80, 98.67, 126.81, 127.12, 129.36, 130.68, 132.85, 135.84, 138.16, 139.18, 166.29, 169.61, 193.41, 208.34, 211.46; high resolution mass spectrum (negative ion FAB) m/z 1141.7 [(M-H); calcd for $C_{63}H_{106}NO_{13}Si_2$: 1141.6].

EXAMPLE 2

31,42-Bis-triethylsilyl ether of 27-hydroxyrapamycin

To a solution of 31,42-bis-triethylsilyl ether of rapamycin (400 mg, 0.35 mmol) in THF (3.5 mL) at −78° C. was added L-Selectride (0.4 mL, 0.4 mmol, 1M in THF) dropwise. After 2 h, the reaction was quenched with H_2O and EtOAc and allowed to warm to room temperature. The aqueous layer was separated and extracted with EtOAc. The organic layers were combined, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified via flash column chromatography using hexane-ethyl acetate (3:1) as eluant to provide 140 mg (35%) of 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin.

IR (KBr) 3300 (s, br), 2950 (s), 2880 (s), 1720 (s), 1640 (s), 1450 (s), 1190 (w), 1100 (s), 1010 (m), 800 (m), 749 (m); ^1H NMR (400 MHz, $CDCl_3$) δ0.47 (m, 6H), 0.49 (m, 6H), 0.57 (m, 1H), 0.81-1.00 (comp m, 27H), 1.01-1.04 (comp m, 6H), 1.14-1.58 (comp m, 16H), 1.60 (d, J=0.83 Hz, 3H), 1.63 (d, J=0.83 Hz, 3H), 1.64-1.82 (comp m, 8H), 2.00 (m, 2H), 2.31 (m, 2H), 2.43 (m, 1H), 2.78 (m, 1H), 2.88 (m, 1H), 3.11 (s, 3H), 3.21, 3.23 (s, rotamers, 3H), 3.37 (m, 3H), 3.40, 3.41 (s, rotamers, 3H), 3.54 (m, 1H), 3.70 (m, 1H), 3.73 (d, J=7.26 Hz, 1H), 3.78 (m, 1H), 4.06 (d, J=7.06 Hz, 1H), 4.81 (s, 1H), 5.02 (m, 1H), 5.23 (d, J=8.72 Hz, 1H), 5.33 (dd, J=0.42, 4.78 Hz, 1H), 5.66 (dd, J=7.15, 15.04 Hz, 1H), 6.00 (d, J=9.75 Hz, 1H), 6.13 (m, 1H), 6.30 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ4.69, 4.99, 5.03, 6.74, 6.82, 10.03, 12.12, 13.78, 14.14, 15.42, 16.16, 20.89, 21.38, 25.37, 27.06, 27.36, 29.69, 31.25, 31.86, 33.20, 33.86, 34.07, 34.15, 34.70, 36.17, 36.37, 38.70, 38.74, 39.71, 42.61, 44.21, 51.17, 55.79, 58.15, 58.22, 67.07, 71.59, 75.70, 79.23, 84.23, 84.85, 98.44, 126.78, 129.51, 130.11, 131.12, 133.31, 135.40, 136.02, 139.27, 167.00, 169.73, 192.86, 212.62; high resolution mass spectrum (negative ion FAB) m/z 1143.7 [(M-H); calcd for $C_{63}H_{108}NO_{13}Si_2$: 1143.6].

EXAMPLE 3

27-Hydroxyrapamycin

31,42-Bis-triethylsilyl ether of 27-hydroxyrapamycin (101 mg, 0.088 mmol) was dissolved in 1.5 mL of

HOAc:THF:H_2O (3:1:1). Additional THF (0.1 mL) was added to effect solution. The reaction was stirred overnight and then diluted with ethyl acetate and washed with $NaHCO_3$. The aqueous layer was back extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified via flash column chromatography using $CHCl_3$:MeOH (95:5) as eluant to provide 57 mg (70%) of 27-Hydroxyrapamycin.

IR (KBr) 3440 (s, br), 2920 (s), 1740 (s), 1650 (s), 1440 (s), 1370 (w), 1190 (w), 1085 (m), 985 (m); ^1H NMR (400 MHz, $CDCl_3$) δ0.68 (m, 1H), 0.83–1.08 (comp m, 15H), 1.16–1.62 (comp m, 12H), 1.66 (s, 3H), 1.68 (s, 3H), 1.71–1.88 (comp m, 8H), 1.98 (m, 2H), 2.14 (m, 3H), 2.28 (m, 2H), 2.39 (m, 1H), 2.66 (s, 1H), 2.84 (m, 1H), 2.94 (m, 1H), 3.13 (s, 3H), 3.28 (d, J=1.18 Hz, 1H), 3.34 (s, 3H), 3.42 (s, 3H), 3.47–3.58 (comp m, 3H), 3.58 (d, J=7.24 Hz, 1H), 3.65 (m, 1H), 3.81 (m, 1H), 4.13 (m, 1H), 4.84 (s, 1H), 4.99 (m, 1H), 5.31 (m, 2H), 5.55 (dd, J=9.0, 24.0 Hz, 1H), 5.94 (d, J=10.5 Hz, 1H), 6.15 (m, 1H), 6.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ10.09, 12.52, 14.03, 15.67, 16.16, 16.22, 20.56, 21.93, 25.27, 26.94, 27.22, 31.22, 31.27, 31.89, 33.22, 33.31, 33.62, 34.00, 35.37, 35.46, 37.99, 38.77, 38.82, 39.03, 40.09, 40.92, 44.22, 51.33, 55.82, 56.62, 60.03, 67.17, 73.65, 73.92, 78.06, 78.89, 84.46, 85.17, 98.42, 126.25, 129.80, 130.31, 131.01, 133.20, 133.73, 135.16, 140.43, 167.06, 170.14, 192.39, 217.19; high resolution mass spectrum (negative ion FAB) m/z 915.3 [(M-H); calcd for $C_{57}H_{80}NO_{13}$: 915.2].

EXAMPLE 4

31,42-Bis-triethylsilyl ether of 27-hydroxyrapamycin-27-ester with acetic acid

To a solution of 31,42-bis-triethylsilyl ether of 27-hydroxyrapamycin (0.74 g, 0.64 mmol) in CH_2Cl_2 (3.2 mL) at 0° C. was added pyridine (0.2 mL, 2.58 mmol) and acetyl chloride (0.092 mL, 1.29 mmol) dropwise. The reaction was held at 0° C. for 30 min, allowed to warm to room temperature, and stirred for 3 h. Additional equivalents of pyridine (0.050 mL, 0.61 mmol) and acetyl chloride (0.023 mL, 0.32 mmol) were added at 0° C. The reaction was again allowed to warm to room temperature and was quenched after an additional 1.5 h with $NaHCO_3$ and diluted with ethyl acetate. The organic layer was separated and washed with 1N HCl, $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed using hexane-ethyl acetate (4:1) as eluant to provide 0.237 g (31%) of 31,42-bis-triethylsilyl ether-27-hydroxyrapamycin-27-ester with acetic acid along with 0.154 g (20%) of recovered starting material.

IR (KBr) 3400 (w, br), 2940 (s), 1740 (s), 1650 (m), 1460 (m), 1240 (s), 1105 (s), 1005 (w), 740 (m); ^1H NMR (400 MHz, $CDCl_3$) δ0.46–0.54 (comp m, 6H), 0.57–0.63 (comp m, 6H), 0.75 (m, 1H), 0.81–0.99 (comp m, 27H), 1.05 (m, 6H), 1.54 (s, 3H), 1.22–1.63 (comp m, 16H), 1.64 (d, J=1.8 Hz, 3H), 1.66–1.98 (comp m, 8H), 1.99 (s, 3H), 2.06 (m, 1H), 2.32 (m, 2H), 2.62 (m, 1H), 2.78 (m, 1H), 2.89 (m, 1H), 3.14 (s, 3H), 3.23 (s, 3H), 3.42 (m, 2H), 3.43 (s, 3H), 3.54 (m, 1H), 3.75 (d, superimposed on m, J=7.2 Hz, 1H), 3.76 (m, 2H), 4.08 (d, J=6.7 Hz, 1H), 4.87 (dd, J=0.41, 4.98 Hz, 1H), 4.99 (m, 1H), 5.03 (m, 1H), 5.20 (d, J=0.4 Hz, 1H), 5.41 (m, 1H), 5.78 (m, 1H), 6.00 (m, 1H), 6.13 (m, 1H), 6.37 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ4.6, 4.9, 6.7, 6.8, 9.9, 14.0, 14.9, 15.3, 16.2, 20.6, 20.8, 20.9, 25.4, 27.3, 27.4, 30.1, 31.3, 31.7, 33.1, 33.3, 33.5, 33.9, 34.0, 34.2, 36.2, 38.2, 38.6, 39.9, 42.6, 44.0, 50.8, 55.6, 58.0, 58.3, 66.9, 73.7, 75.6, 75.9, 76.4, 79.1, 84.1, 84.4, 98.2, 126.6, 129.6, 129.9, 130.0, 133.6, 134.5, 135.9, 139.0, 167.1, 169.3, 170.5, 191.6, 212.0; high resolution mass spectrum (negative ion FAB) m/z 1185.7 [(M-H); calcd for $C_{65}H_{110}NO_{14}Si_2$: 1185.6].

EXAMPLE 5

27-Hydroxyrapamycin-27-ester with acetic acid

31,42-bis-triethylsilyl ether-27-hydroxyrapamycin-27-ester with acetic acid (0.16 g, 0.13 mmol) was dissolved in 2.5 mL of a 3:1:1 solution of HOAc:THF:H_2O. The reaction was stirred overnight and was then quenched with $NaHCO_3$ and diluted with ethyl acetate. The organic layer was separated and washed with $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed using CH_2Cl_2:MeOH (20:1) as eluant followed by HPLC (70:30 hexane:ethyl acetate gradient over 60 min to 100% ethyl acetate) to provide 0.050 g (40%) of 27-hydroxyrapamycin-27-ester with acetic acid.

^1H NMR (400 MHz, $CDCl_3$) δ0.68 (m, 1H), 0.95–1.04 (comp m, 15H), 1.13–1.69 (comp m, 18H), 1.59 (s, 3H), 1.66 (d, J=5.6 Hz, 3H), 1.78–1.98 (comp m, 9H), 2.02 (s, 3H), 2.30 (m, 2H), 2.68 (m, 1H), 2.85 (m, 1H), 2.95 (m, 1H), 3.13 (s, 3H), 3.37 (s, 3H), 3.43 (s, superimposed on m, 3H), 3.43 (m, 2H), 3.59–3.70 (comp m, 3H), 3.79 (m, 1H), 4.09 (d, J=7.9 Hz, 1H), 4.80 (m, 2H), 5.17 (s, 1H), 5.25 (d, J=10.0 Hz, 1H), 5.35 (d, J=5.3 Hz, 1H), 5.76 (dd, J=8.9, 19.8 Hz, 1H), 5.90 (d, J=9.1 Hz, 1H), 6.14 (m, 1H), 6.36 (m, 2H); high resolution mass spectrum (negative ion FAB) m/z 957.2 [(M-H); calcd for $C_{53}H_{82}NO_{14}$: 957.5].

Anal. Calcd for $C_{53}H_{83}NO_{14}\bullet 0.1Et_2O$: C, 65.9 H, 8.66 N, 1.45. Found: C, 65.9 H, 8.72 N, 1.34.

The following representative compounds or pharmaceutically acceptable salts thereof could be readily prepared based on the methodology described in this disclosure.

27-Hydroxyrapamycin-27-ester with benzoic acid
27-Hydroxyrapamycin-27-ester with phenylacetic acid
27-Hydroxyrapamycin-27-ester with pyridine-2-carboxylic acid
27-Hydroxyrapamycin-27-ester with trifluoroacetic acid
27-Hydroxyrapamycin-27-ester with 3,3,3-trifluoropropanoic acid
27-Hydroxyrapamycin-27-ester with difluoroacetic acid
27-Hydroxyrapamycin-27-ester with pentafluoropropionic acid
27-Hydroxyrapamycin-27-ester with 4-(dimethylamino)-4-oxobutanoic acid
27-Hydroxyrapamycin-27-ester with 4-oxo-4-[[2-(2-pyridinyl)ethyl]amino]butanoic acid
27-Hydroxyrapamycin-27-ester with 2-[2-[(3-carboxy-1-oxopropyl)amino]ethyl]-1-methyl-pyridinium iodide
27-Hydroxyrapamycin-27-ester with (4-fluorophenyl)-carbamic acid
27-Hydroxyrapamycin-27-ester with phenylcarbamic acid
27-Hydroxyrapamycin-27-ester with 4-[(trifluoromethyl)phenyl]carbamic acid
27-Hydroxyrapamycin-27-ester with (4-nitrophenyl)-carbamic acid
27-Hydroxyrapamycin-27-ester with (4-methylphenyl)carbamic acid

27-Hydroxyrapamycin-27-ester with (2,4-difluorophenyl)carbamic acid

27-Hydroxyrapamycin-27-ester with N-[(1,1-dimethylethoxy)carbonyl]-glycylglycine

27-Hydroxyrapamycin-27-ester with N-[(1,1-dimethylethoxy)carbonyl]-N-methylglycine

27-Hydroxyrapamycin-27-ester with 5-(1,1-dimethylethoxy)-2-[[(1,1-dimethylethoxy)carbonyl]amino]-5-oxopentanoic acid

27-Hydroxyrapamycin-27-ester with 2-[[(1,1-dimethylethoxy)carbonyl]amino]-4-oxo-4-(phenylmethoxy)-butanoic acid

27-Hydroxyrapamycin-27-ester with 3-[[(1,1-dimethylethoxy)carbonyl]amino]-4-oxo-4-(phenylmethoxy)-butanoic acid

27-Hydroxyrapamycin-27-ester with 5-(1,1-dimethyloxy)-4-[[(1,1-dimethylethoxy)carbonyl]amino]-5-oxopentanoic acid

27-Hydroxyrapamycin-27-ester with N^{α}, N^{ϵ}-bis[(1,1-dimethylethoxy)carbonyl]-L-lysine

27-Hydroxyrapamycin-27-ether with (1-methoxy-1-methyl)ethanol

27-Hydroxyrapamycin-27-ether with (2-(trimethylsilyl-)ethoxy)methanol

27-Hydroxyrapamycin-27-ester with N,N-dimethylglycine

27-Hydroxyrapamycin-27-ester with 3-(N,N-diethylamino)propionic acid

27-Hydroxyrapamycin-27-ester with 4'-(N-pyrrolidino)butyric acid

27-Hydroxyrapamycin-27-ester with phenylsulfonylcarbamic acid

27-Hydroxyrapamycin-27-ester with (4-chlorophenylsulfonyl)carbamic acid

27-Hydroxyrapamycin-27-ester with (3-methylphenylsulfonyl)carbamic acid

27-Hydroxyrapamycin-27-ester with 5-(dimethylamino)-1-naphthalensulfonic acid

27-Hydroxyrapamycin-27-ester with 4-methylbenzenesulfonic acid

27-Hydroxyrapamycin-27-ester with 2-thiophenesulfonic acid

27-Hydroxyrapamycin-27-ester with 4-[[4-(dimethylamino)phenyl]aza]benzenesulfonic acid

27-Hydroxyrapamycin-27-ester with 1-naphthalenesulfonic acid

27-Hydroxyrapamycin-27-ester with 8-quinolinsulfonic acid

27-Hydroxyrapamycin-27-ester with methanesulfonic acid

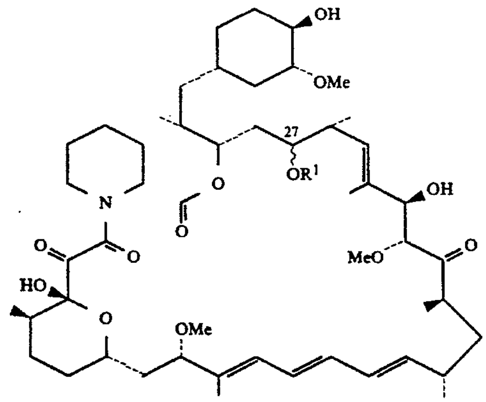
27-Hydroxyrapamycin-27-ester with 2,2,2-trifluoroethanesulfonic acid

27-Hydroxyrapamycin-27-ester with [(methoxycarbonyl)amino]sulfonic acid

What is claimed is:

1. A compound of the formula

II



wherein
R^1 is

$$-\overset{O}{\overset{\|}{C}}R^2$$

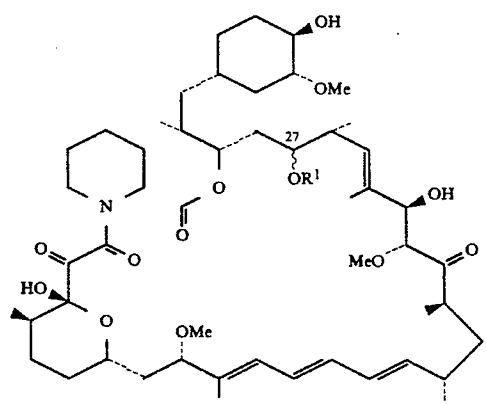
and
R^2 is alkyl of 1–10 carbon atoms, arylalkyl of 7–10 carbon atoms, or aryl wherein the aryl group may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1–6 carbon atoms per alkyl group, alkylthio of 1–6 carbon atoms, $-SO_3H$, $-PO_3H$, and $-CO_2H$;

or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1 which is 27-hydroxyrapamycin-27-ester with acetic acid.

3. A compound of the formula

III



wherein
R^1 is

$$-X-\overset{O}{\overset{\|}{C}}-NR^3R^4;$$

and

R^2 is a mono-, di-, poly-, or per-fluorinated alkyl group of 1–10 carbon atoms.

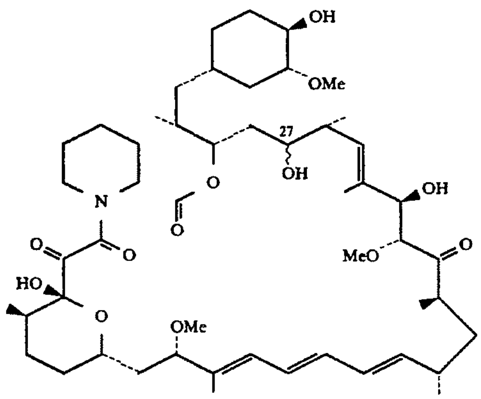
4. A compound of the formula

$$-\overset{O}{\overset{\|}{C}}R^2$$

wherein

R^1 is

IV



R^2 is

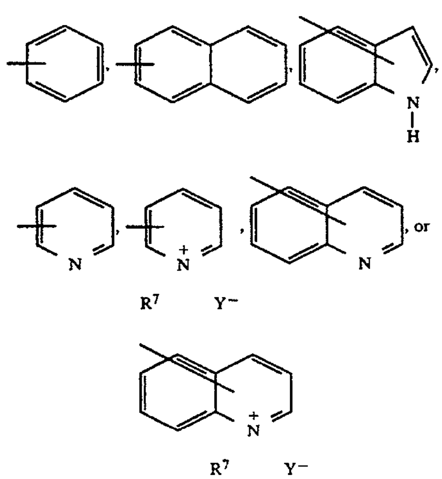
$$-\overset{O}{\overset{\|}{C}}R^2;$$

X is $-(CH_2)_m-$ or $-Ar-$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–12 carbon atoms, $-(CH_2)_n-Ar$, $-(CH_2)_p-NR^5R^6$, or $-(CH_2)_p-N^+R^5R^6R^7Y^-$;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–12 carbon atoms, or $-(CH_2)_n-Ar$;

Ar is an optionally mono- or di-substituted group selected from



in which the optional substituents are selected from the group consisting of alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7

carbon atoms, or perfluoroalkyl of 1–6 carbon atoms;

R^7 is alkyl of 1–6 carbon atoms;

Y is a halide, sulfate, phosphate, or p-toluenesulfonate anion;

m=1–6;

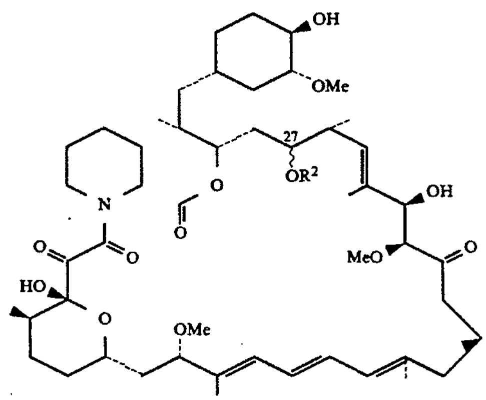
n=1–6;

p=1–6;

or a pharmaceutically acceptable salt thereof.

5. A compound of the formula

VI



wherein

R^2 is

$$-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^3}{\underset{|}{CH}}(CH_2)_n\underset{R^4}{\underset{|}{N}}]_pCO_2R^5;$$

R^3 is hydrogen, alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, $-(CH_2)_qCO_2R^6$, $-(CH_2)_rNR^7CO_2R^8$, carbamylalkyl of 2–3 carbon atoms, aminoalkyl of 1–4 carbon atoms, hydroxyalkyl of 1–4 carbon atoms, guanylalkyl of 2–4 carbon atoms, mercaptoalkyl of 1–4 carbon atoms, alkylthioalkyl of 2–6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazoylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1–6 carbon atoms, alkoxy of 1–6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, or $-CO_2H$;

R^4 and R^7 are each, independently, hydrogen, alkyl of 1–6 carbon atoms, or arylalkyl of 7–10 carbon atoms;

R^5, R^6, and R^8 are each, independently, alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1–6 carbon atoms, alkoxy of 1–6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, or $-CO_2H$;

m is 0–4;

n is 0–4;

p is 1–2;

q is 0–4;

r is 0–4;

wherein R^3, R^4, m, and n are independent in each of the

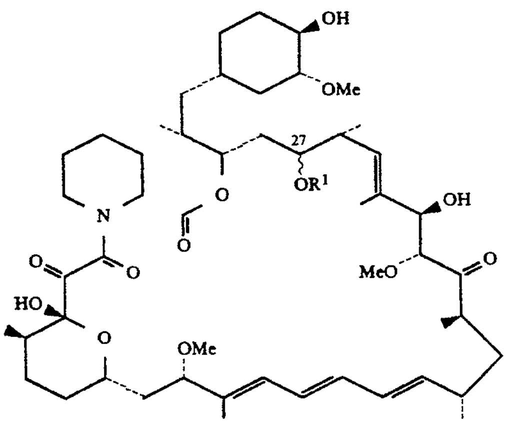
$$[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^3}{\underset{|}{CH}}(CH_2)_n\underset{R^4}{\underset{|}{N}}]$$

subunits when p=2;

or a pharmaceutically acceptable salt thereof.

6. A compound of the formula

VII



wherein

R^1 is alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, $—CH_2YX$, $—C(CH_3)_2YX$, $—CH(CH_3)YX$, or L;

Y is O or S;

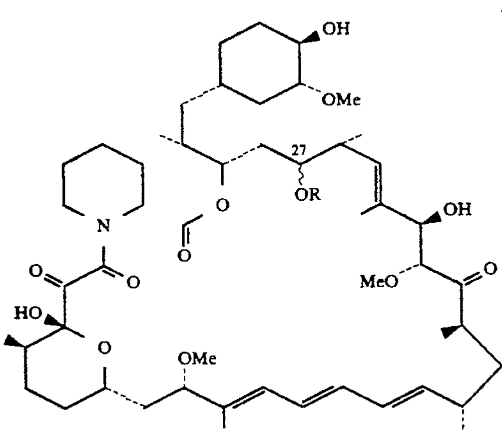
X is $—CH_3$, $—(CH_2)_nCH_3$, $—CH_2Ar$, $—(CH_2)_2OCH_3$, $—CH_2CCl_3$, $—CH(CH_3)_2$, or $—CH_2CH_2SiMe_3$;

L is tetrahydrofuran-2-yl, tetrahydrothiophen-2-yl, tetrahydrothiopyran-2-yl, tetrahydropyran-2-yl, 4-methoxytetrahydropyran-2-yl, 4-methoxytetrahydrothiopyran-2-yl, or 4-methoxytetrahydrothiopyran-2-yl S,S dioxide; and

n=1-5.

7. A compound of the formula

VIII



wherein

R is

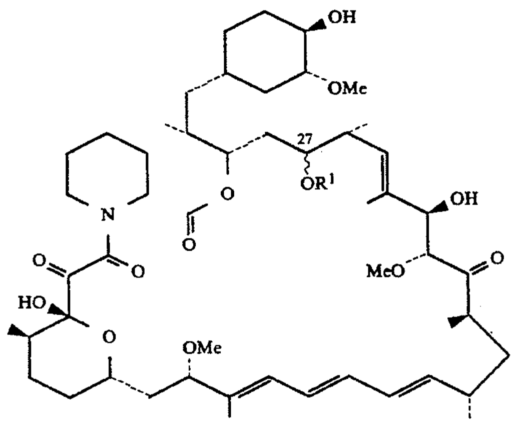
$$—\overset{O}{\overset{\|}{C}}(CH_2)_mNR^1R^2;$$

R^1 and R^2 are each hydrogen or alkyl of 1-3 carbon atoms or R^1 and R^2 together with the nitrogen to which they are attached form a saturated heterocyclic ring having 4–5 carbon atoms; and

m=1-3 or a pharmaceutically acceptable salt thereof.

8. A compound of the formula

IX



wherein

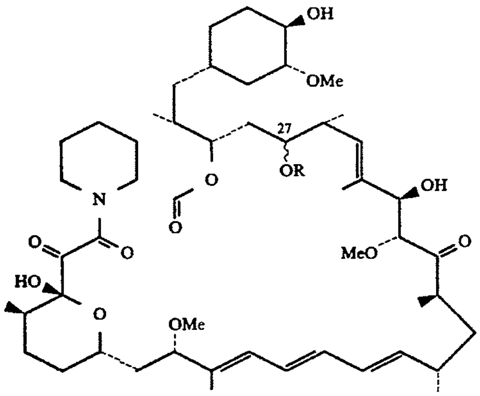
R^1 is $—CONHSO_2—Ar$; and

Ar is phenyl, naphthyl, pyridyl, quinolyl, isoquinolyl, quinoxalyl, thienyl, thionaphthyl, furyl, benzofuryl, benzodioxyl, benzoxazolyl, benzoisoxazolyl, or benzodioxolyl; wherein the Ar group may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1–6 carbon atoms per alkyl group, alkylthio of 1–6 carbon atoms, $—SO_3H$, $—PO_3H$, and $—CO_2H$;

or a pharmaceutically acceptable salt thereof when the Ar group contains a basic nitrogen or when the Ar group is substituted by dialklyamino of 1–6 carbon atoms per alkyl group, $—SO_3H$, $—PO_3H$, or $—CO_2H$;

or a pharmaceutically acceptable salt thereof.

9. A compound of the formula



X

wherein

R is $-SO_2R^1$;

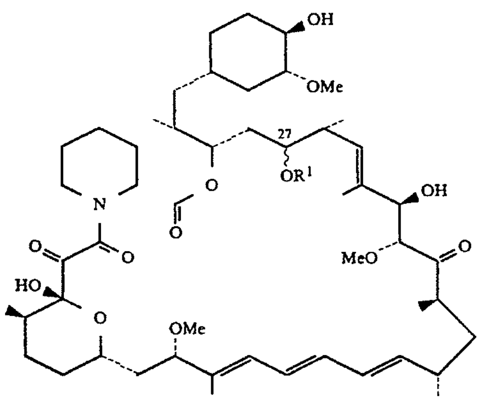
R^1 is alkyl, alkenyl, alkynyl containing 1 to 6 carbon atoms; or an aromatic moiety selected from the group consisting of phenyl and naphthyl or a heterocyclic moiety selected from the group consisting of thiophenyl and quinolinyl; or $-NHCOR^2$; and

R^2 is lower alkyl containing 1 to 6 carbon atoms;

or a pharmaceutically acceptable salt thereof.

10. A method of inducing immunosuppression which comprises administering an immunosuppressive effective amount of a compound of formula II,

II



wherein

R^1 is

$$-\overset{\overset{\displaystyle O}{\|}}{C}R^2$$

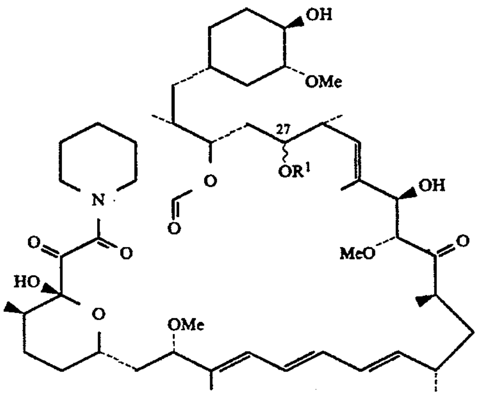
and

R^2 is alkyl of 1-10 carbon atoms, arylalkyl of 7-10 carbon atoms, or aryl wherein the aryl group may be optionally mono-, di-, or tri- substituted with a group selected from alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, alkoxy of 1-6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1-6 carbon atoms per alkyl group, alkylthio of 1-6 carbon atoms, $-SO_3H$, $-PO_3H$, and $-CO_2H$;

or a pharmaceutically acceptable salt thereof;

or of formula III,

III



wherein

R^1 is

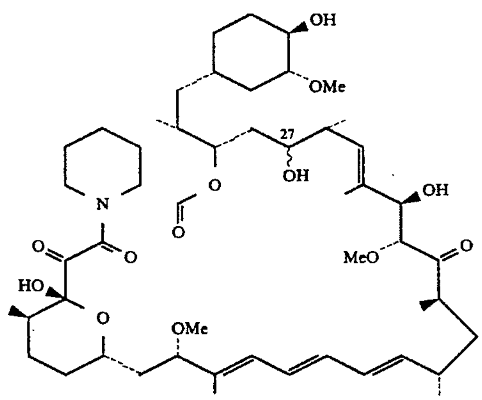
$$-\overset{\overset{\displaystyle O}{\|}}{C}R^2$$

and

R^2 is a mono-, di-, poly-, or per-fluorinated alkyl group of 1-10 carbon atoms;

or of formula IV,

IV



wherein

R^1 is

$$-\overset{\overset{\displaystyle O}{\|}}{C}R^2;$$

R^2 is

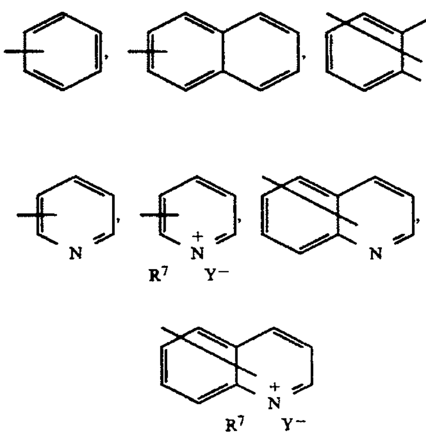
$$-X-\overset{\overset{\displaystyle O}{\|}}{C}-NR^3R^4;$$

X is $-(CH_2)_m-$ or $-Ar-$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1-12 carbon atoms, —$(CH_2)_n$—Ar, —$(CH_2)_p$—NR^5R^6, or —$(CH_2)_p$—$N^+R^5R^6R^7Y^-$;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1-12 carbon atoms, or —$(CH_2)_n$—Ar;

Ar is an optionally mono- or di- substituted group selected from



in which the optional substituents are selected from the group consisting of alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, alkoxy of 1-6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, or perfluoroalkyl of 1-6 carbon atoms;

R^7 is alkyl of 1-6 carbon atoms;

Y is a halide, sulfate, phosphate, or p-toluenesulfonate anion;

m=1-6;

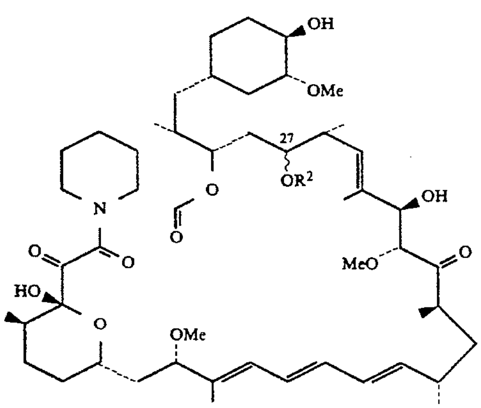
n=1-6;

p=1-6;

or a pharmaceutically acceptable salt thereof;

or of formula VI,

VI



wherein

R^2 is

$$-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^3}{\underset{|}{C}H}(CH_2)_n\underset{R^4}{\underset{|}{N}}]_pCO_2R^5;$$

R^3 is hydrogen, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, —$(CH_2)_qCO_2R^6$, —$(CH_2)_rNR^7CO_2R^8$, carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazoylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or —CO_2H;

R^4 and R^7 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms;

R^5, R^6, and R^8 are each, independently, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or —CO_2H;

m is 0-4;

n is 0-4;

p is 1-2;

q is 0-4;

r is 0-4;

wherein

R^3, R^4, m, and n are independent in each of the

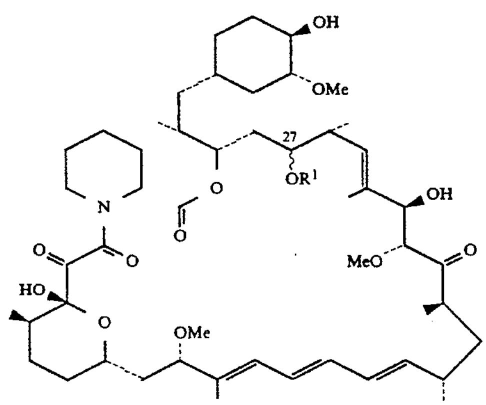
$$[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^3}{\underset{|}{C}H}(CH_2)_n\underset{R^4}{\underset{|}{N}}]$$

subunits when p=2;

or a pharmaceutically acceptable salt thereof;

or of formula VII,

VII



wherein

R^1 is alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, $-CH_2YX$, $-C(CH_3)_2YX$, $-CH(CH_3)YX$, or L;

Y is O or S;

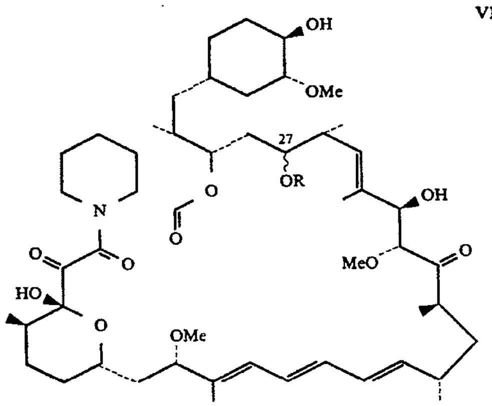
X is $-CH_3$, $-(CH_2)_nCH_3$, $-CH_2Ar$, $-(CH_2)_2OCH_3$, $-CH_2CCl_3$, $-CH(CH_3)_2$, or $-CH_2CH_2SiMe_3$;

L is tetrahydrofuran-2-yl, tetrahydrothiophen-2-yl, tetrahydrothiopyran-2-yl, tetrahydropyran-2-yl, 4-methoxytetrahydropyran-2-yl, 4-methoxytetrahydrothiopyran-2-yl, or 4-methoxytetrahydrothiopyran-2-yl S,S dioxide; and

$n=1-5$;

or of formula VIII,

VIII



wherein

R is

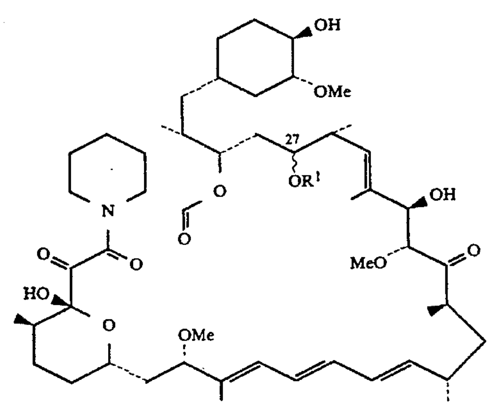
$$-\overset{O}{\overset{\|}{C}}(CH_2)_mNR^1R^2;$$

R^1 and R^2 are each hydrogen or alkyl of 1-3 carbon atoms or R^1 and R^2 together with the nitrogen to which they are attached form a saturated heterocyclic ring having 4-5 carbon atoms; and

$m=1-3$ or a pharmaceutically acceptable salt thereof;

or of formula IX,

IX



wherein

R^1 is $-CONHSO_2-Ar$; and

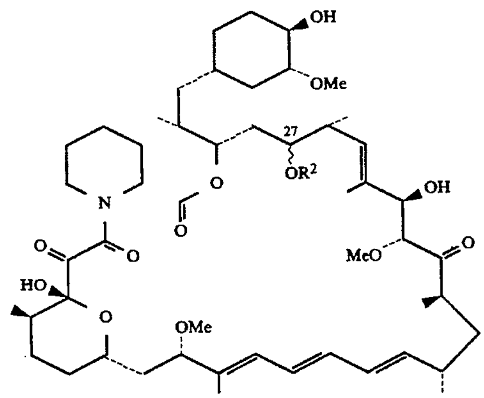
Ar is phenyl, naphthyl, pyridyl, quinolyl, isoquinolyl, quinoxalyl, thienyl, thionaphthyl, furyl, benzofuryl, benzodioxyl, benzoxazolyl, benzoisoxazolyl, or benzodioxolyl; wherein the Ar group may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, alkoxy of 1-6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1-6 carbon atoms per alkyl group, alkylthio of 1-6 carbon atoms, $-SO_3H$, $-PO_3H$, and $-CO_2H$;

or a pharmaceutically acceptable salt thereof when the Ar group contains a basic nitrogen or when the Ar group is substituted by dialkylamino of 1-6 carbon atoms per alkyl group, $-SO_3H$, $-PO_3H$, or $-CO_2H$;

or a pharmaceutically acceptable salt thereof;

or of formula X,

X



wherein

R is $-SO_2R^1$;

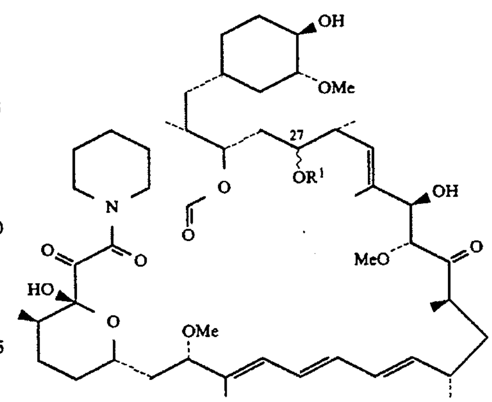
R^1 is alkyl, alkenyl, alkynyl containing 1 to 6 carbon atoms; or an aromatic moiety selected from the group consisting of phenyl and naphthyl or a heterocyclic moiety selected from the group consisting of thiophenyl and quinolinyl; or $-NHCOR^2$; and

R^2 is lower alkyl containing 1 to 6 carbon atoms;

or a pharmaceutically acceptable salt thereof.

11. A pharmaceutical composition which comprises an effective amount of a compound of formula II,

II



wherein

R^1 is

$$-\overset{\overset{\displaystyle O}{\|}}{C}R^2$$

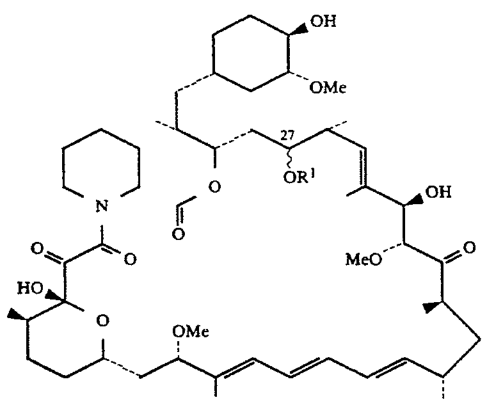
and

R^2 is alkyl of 1–10 carbon atoms, arylalkyl of 7–10 carbon atoms, or aryl wherein the aryl group may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1–6 carbon atoms per alkyl group, alkylthio of 1–6 carbon atoms, $-SO_3H$, $-PO_3H$, and $-CO_2H$;

or a pharmaceutically acceptable salt thereof;

or of formula III,

III



wherein

R^1 is

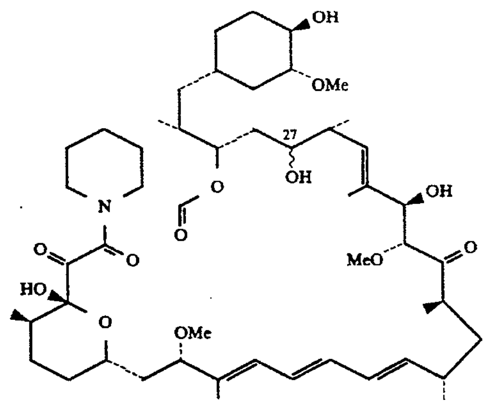
$$-\overset{\overset{\displaystyle O}{\|}}{C}R^2$$

and

R^2 is a mono-, di-, poly-, or per-fluorinated alkyl group of 1–10 carbon atoms;

or of formula IV,

IV



wherein

R^1 is

$$-\overset{\overset{\displaystyle O}{\|}}{C}R^2;$$

R^2 is

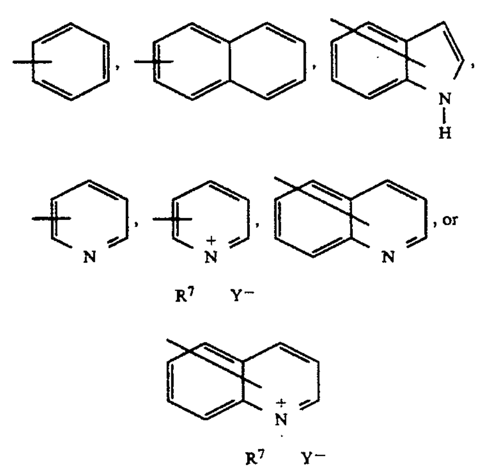
$$-X-\overset{\overset{\displaystyle O}{\|}}{C}-NR^3R^4;$$

X is $-(CH_2)_m-$ or $-Ar-$;

R^3 and R^4 are each, independently, hydrogen, alkyl of 1–12 carbon atoms, $-(CH_2)_n-Ar$, $-(CH_2)_p-NR^5R^6$, or $-(CH_2)_p-N^+R^5R^6R^7Y^-$;

R^5 and R^6 are each, independently, hydrogen, alkyl of 1–12 carbon atoms, or $-(CH_2)_n-Ar$;

Ar is an optionally mono- or di-substituted group selected from



in which the optional substituents are selected from the group consisting of alkyl of 1–6 carbon atoms, arylalkyl of 7–10 carbon atoms, alkoxy of 1–6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2–7 carbon atoms, or perfluoroalkyl of 1–6 carbon atoms;

R^7 is alkyl of 1-6 carbon atoms;

Y is a halide, sulfate, phosphate, or p-toluenesulfonate anion;

m=1-6;

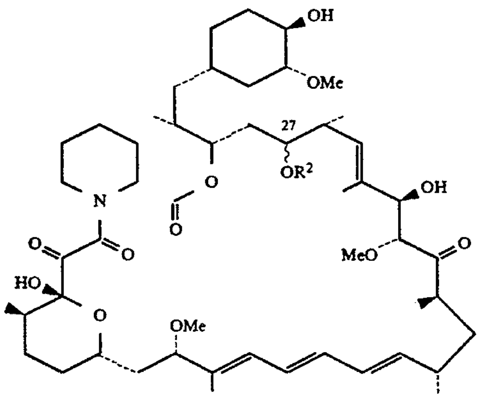
n=1-6;

p=1-6;

or a pharmaceutically acceptable salt thereof;

or of formula VI,

VI



wherein

R^2 is

$$-[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^3}{\underset{|}{C}}H(CH_2)_n\underset{R^4}{\underset{|}{N}}]_pCO_2R^5;$$

R^3 is hydrogen, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, $-(CH_2)_qCO_2R^6$, $-(CH_2)_rNR^7CO_2R^8$, carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indoylmethyl, hydroxyphenylmethyl, imidazoylmethyl or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or $-CO_2H$;

R^4 and R^7 are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms;

R^5, R^6, and R^8 are each, independently, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, fluorenylmethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or $-CO_2H$;

m is 0-4;

n is 0-4;

p is 1-2;

q is 0-4;

r is 0-4;

wherein R^3, R^4, m, and n are independent in each of the

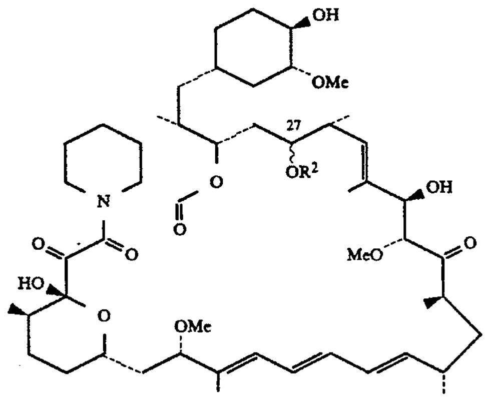
$$[\overset{O}{\overset{\|}{C}}(CH_2)_m\underset{R^3}{\underset{|}{C}}H(CH_2)_n\underset{R^4}{\underset{|}{N}}]$$

subunits when p=2;

or a pharmaceutically acceptable salt thereof;

or of formula VII,

VII



wherein

R^1 is alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, $-CH_2YX$, $-C(CH_3)_2YX$, $-CH(CH_3)YX$, or L;

Y is O or S;

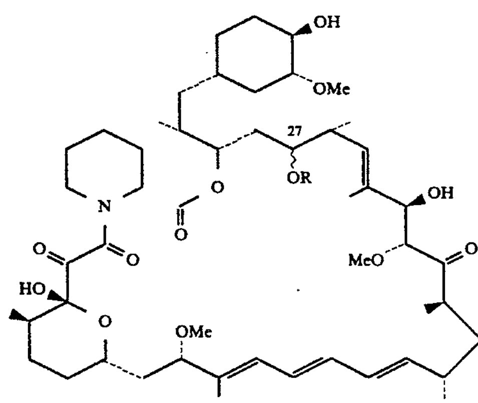
X is $-CH_3$, $-(CH_2)_nCH_3$, $-CH_2Ar$, $-(CH_2)_2OCH_3$, $-CH_2CCl_3$, $-CH(CH_3)_2$, or $-CH_2CH_2SiMe_3$;

L is tetrahydrofuran-2-yl, tetrahydrothiophen-2-yl, tetrahydrothiopyran-2-yl, tetrahydropyran-2-yl, 4-methoxytetrahydropyran-2-yl, 4-methoxytetrahydrothiopyran-2-yl, or 4-methoxytetrahydrothiopyran-2-yl S,S dioxide; and

n=1-5;

or of formula VIII,

VIII



wherein

R is

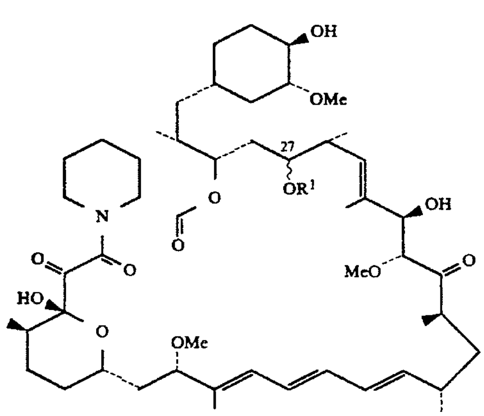
$$-\overset{O}{\overset{\|}{C}}(CH_2)_mNR^1R^2;$$

R^1 and R^2 are each hydrogen or alkyl of 1-3 carbon atoms or R^1 and R^2 together with the nitrogen to which they are attached form a saturated heterocyclic ring having 4-5 carbon atoms; and

$m = 1-3$ or a pharmaceutically acceptable salt thereof;

or of formula IX,

IX



wherein

R^1 is $-CONHSO_2-Ar$; and

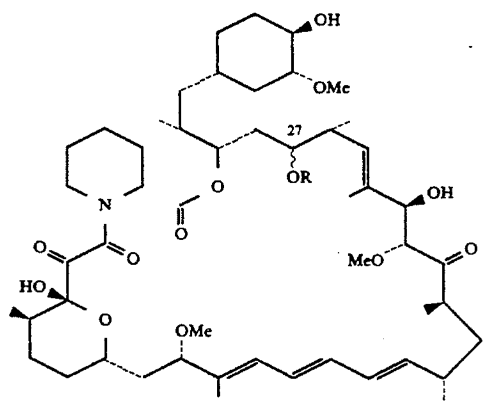
Ar is phenyl, naphthyl, pyridyl, quinolyl, isoquinolyl, quinoxalyl, thienyl, thionaphthyl, furyl, benzofuryl, benzodioxyl, benzoxazolyl, benzoisoxazolyl, or benzodioxolyl; wherein the Ar group may be optionally mono-, di-, or tri-substituted with a group selected from alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, alkoxy of 1-6 carbon atoms, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, dialkylamino of 1-6 carbon atoms per alkyl group, alkylthio of 1-6 carbon atoms, $-SO_3H$, $-PO_3H$, and $-CO_2H$;

or a pharmaceutically acceptable salt thereof when the Ar group contains a basic nitrogen or when the Ar group is substituted by dialkylamino of 1-6 carbon atoms per alkyl group, $-SO_3H$, $-PO_3H$, or $-CO_2H$;

or a pharmaceutically acceptable salt thereof;

or of formula X,

X



wherein

R is $-SO_2R^1$;

R^1 is alkyl, alkenyl, alkynyl containing 1 to 6 carbon atoms; or an aromatic moiety selected from the group consisting of phenyl and naphthyl or a heterocyclic moiety selected from the group consisting of thiophenyl and quinolinyl; or $-NHCOR^2$; and

R^2 is lower alkyl containing 1 to 6 carbon atoms;

or a pharmaceutically acceptable salt thereof,

and a pharmaceutically acceptable carrier.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,256,790

Page 1 of 8

DATED : October 26, 1993

INVENTOR(S) : Frances C. Nelson

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

The title page should be deleted and substitute therefor the attached title page.

Columns 11,12, 21,22,23,24,27,28,31,32,33, and 34, should be deleted and substitute therefore the attached pages.

Signed and Sealed this

Fourteenth Day of February, 1995

Attest:

Bruce Lehman

BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks